VOLUME 45 DECEMBER 1955 NUMBER 12

# Phytopathology

## An International Journal

*Official Organ of*

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

UNIVERSITY OF MICHIGAN
JAN 16
SCIENCE LIBRARY

CONTENTS

Heterocaryosis as a basis for variation in Puccinia graminis var. tritici
R. R. Nelson, Roy D. Wilcoxson, and J. J. Christensen 639

Temperature and the geographical distribution of chrysanthemum rust
Colin E. Campbell and A. W. Dimock 644

Relation of soft rot development to protective barriers in Irish potato slices
W. L. Smith, Jr., and Helen F. Smart 649

Host-penetration and pathological histology in gray leaf spot of tomato
Urban L. Diener 654

A study on the mode of action of the wildfire toxin — Armin C. Braun 659

Vector-feeding and plant-tissue relationships in the transmission of the Wisconsin pea streak virus — C. B. Skotland and D. J. Hagedorn 665

Physiology and nature of disease development in winter crown rot of alfalfa
J. B. Lebeau and J. G. Dickson 667

Effect of brief exposures at 40°C on germination of sporangia of Phytophthora infestans
Carlton F. Taylor, John J. Smoot, D. O. Quinn, R. A. Rhode, and Edward E. Elliott 673

Some effects of inorganic nutrients on the growth and pathogenicity of five fungal pathogens of gladiolus — B. H. Marshall, Jr. 676

Host-parasite interaction in flax rust—its genetics and other implications — H. H. Flor 680

The relation of Valsa kunzei to cankers on conifers — Alma M. Waterman 686

Report and abstracts of the thirty-seventh annual meeting of the Pacific Division of the American Phytopathological Society — 692

News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

# Rohm & Haas **fungicides** available for research

The Rohm & Haas Company, manufacturers of DITHANE and many other outstanding agricultural chemicals, is happy to offer phytopathologists and other workers in the field, test quantities of many different products for experimental use.

Principal Rohm & Haas agricultural fungicides are:

**KARATHANE WD**—a 25% active ingredient concentrate, suitable for spraying or preparation of dusts, based on dinitro 1-methyl heptyl phenyl crotonate. KARATHANE is highly effective as an eradicant and protectant against representative species of every genus of powdery mildew on fruits, vegetables and ornamentals. An active miticide, it is particularly effective on brown almond mite. Possessing some activity against a variety of diseases, insects and mites, it merits broad consideration in your research work.

**DITHANE Z-78 (zineb)**—a 65% ethylene bisdithiocarbamate powder suitable for spraying or dust blending. A fungicide, it controls many diseases on a wide variety of crops. Its latest field of application, and one which deserves further study, is in the control of soil fungi responsible for damping-off and "sore-shin" on cotton seedlings.

**DITHANE M-22 (maneb)**—70% manganese ethylene bisdithiocarbamate. A powder suitable for spraying or dust blending, it is particularly effective in the control of tomato diseases.

**DITHANE D-14 (nabam)**—19% sodium ethylene bisdithiocarbamate. A water-soluble liquid usually combined with a soluble metallic salt—zinc sulfate or ferric sulfate or manganese sulfate for the preparation of tank-mix fungicides. One development with this product creating considerable interest, is its application as a fungicide when mixed with complex salts of zinc, manganese and iron.

These and many other Rohm & Haas products for use in agriculture have found a wide market. Others are in the developmental stage.

Research workers who would like to have further information or adequate samples of these chemicals for testing purposes are invited to write directly to our main office.

CHEMICALS FOR AGRICULTURE

**ROHM & HAAS COMPANY**

WASHINGTON SQUARE, PHILADELPHIA 5, PA.

*Representatives in principal foreign countries*

DITHANE *and* KARATHANE *are trademarks, Reg. U. S. Pat. Off. and in principal foreign countries.*

# PHYTOPATHOLOGY

**An International Journal**

**Official Organ of The American Phytopathological Society**

EDITORS

A. F. Ross, Editor-in-Chief, Cornell University, Ithaca, New York

C. S. Holton, State College of Washington, Pullman, Washington

Ralph M. Caldwell, Purdue University, Lafayette, Indiana

S. E. A. McCallan, Boyce Thompson Institute, Yonkers 3, N. Y.

ASSOCIATE EDITORS

Vincent W. Cochrane, Wesleyan University, Middletown, Connecticut

Frank P. McWhorter, Oregon State College, Corvallis, Oregon

Earle W. Hansen, University of Wisconsin, Madison, Wisconsin

Edward O. Olson, Citrus Rootstock Investigations, Box 144, Weslaco, Texas

M. T. Hilborn, Agricultural Experiment Station, Orono, Maine

Mortimer P. Starr, University of California, Davis, California

W. J. Hooker, Michigan State College, East Lansing, Michigan

Albert L. Taylor, Plant Industry Station, Beltsville, Maryland

W. F. Jeffers, College Heights, Hyattsville, Maryland

Arthur Kelman, North Carolina State College, Raleigh, North Carolina

R. P. True, West Virginia University, Morgantown, West Virginia

C. E. Yarwood, University of California, Berkeley, California

BUSINESS MANAGEMENT

Saul Rich, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

L. J. Alexander, Advertising Manager, Agricultural Experiment Station, Wooster, Ohio.

NOTICES

Send communications regarding subscriptions and back numbers to Saul Rich, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

**Subscription price.** $10.00 per year, domestic and foreign. Single current numbers (within 60 days of publication), $1.00. The journal has been issued monthly beginning with Volume 8. **Separate Numbers** may be purchased from back volumes at $1.00 per copy. Complete unbound volumes, 1 through 7, when available, $6.00 each; 8 through 41, as available, $10.00 each. **Thirty-Year Index** (Vols. 1–30, 1911–1940), bound: $7.50, domestic and foreign. **Ten-Year Index** (Vols. 31–40, 1941–1950), paper cover, $3.50. **Lost Numbers.** Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of $1.00 will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk. **Separates.** No gratis copies supplied. Quotation is sent with proof.

**Advertisements.** Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisements is assumed by this Journal or by The American Phytopathological Society.

**Manuscripts and editorial correspondence** should be sent to the Editor-in-Chief. At least 1 of the authors must be a member of the American Phytopathological Society. Type on only 1 side of sheet; double-space all parts of manuscript, including footnotes, citations, and legends. For papers longer than 1 printed page, the article should begin with a brief, concise summary. Number footnotes consecutively with arabic numerals, except in tables where lower case letters are used. Give each table a heading stating its contents. Make references to literature as footnotes only when 5 or fewer citations are given. Name and address of the institution where the work was done should precede "Literature Cited." List references alphabetically in "Literature Cited" and number consecutively throughout text as well as in list of citations. Authors are advised to read "Preparing manuscripts for Phytopathology" by W. C. Price, published in Phytopathology 44: 667-674, 1954, as well as current issues of Phytopathology.

**Illustrations** must be limited in number and should be sharp and clear. One-half the cost of engraving in excess of $16.00, the approximate cost of a single full page cut or of two 3 in. x 4½ in. cuts, are charged to the author. No charge is made for line drawings that substitute for tables unless drawings and tables together exceed 30 per cent of the length of the article. Illustrations are run as text figures designated by arabic numerals. Units of a composite illustration are designated by letters. Arrange full-page figures to conform to journal proportions (6⅛ x 8½ in.), making due allowance for reductions. Specific reference to each illustration must be made in the text, and each must be accompanied by an adequate legend, typed double-space on a separate paper and not on the illustration.

**The responsibility** for statements, whether of fact or opinion, rests entirely with the writers thereof.

# The American Phytopathological Society

FOUNDED 1908. INCORPORATED 1915.

## THE A. P. S. COUNCIL

President—James H. Jensen, Provost, Iowa State College, Ames, Iowa
Vice-President—George W. Fischer, Washington State College, Pullman, Washington
Secretary—Glenn S. Pound, University of Wisconsin, Madison 6, Wisconsin
Treasurer—Saul Rich, Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Connecticut
Editor-in-Chief—A. F. Ross, Cornell University, Ithaca, New York
President-Elect—Helen Hart, University of Minnesota, St. Paul 1, Minnesota
Retiring President—George F. Weber, University of Florida, Gainesville, Florida

Councilors:

J. L. Allison, North Carolina State College, Raleigh, North Carolina
E. C. Blodgett, Irrigation Experiment Station, Prosser, Washington
W. D. McClellan, U. S. Department of Agriculture, Beltsville, Maryland
Paul R. Miller, Plant Disease Survey, U. S. Department of Agriculture, Beltsville, Maryland
E. G. Rex, New Jersey Department of Agriculture, Trenton, New Jersey
J. R. Shay, Purdue University, Lafayette, Indiana
W. C. Snyder, University of California, Berkeley, California

## DIVISIONS OF THE SOCIETY

Northeastern: F. O. Holmes, Rockefeller Institute for Medical Research, New York, New York, *Pres.;* B. H. Davis, Rutgers University, New Brunswick, New Jersey, *Vice-Pres.;* B. A. Friedman, U.S.D.A. Market Pathology Laboratory, New York, New York, *Sec.-Treas.*

North Central: C. O. Johnston, Kansas State College, Manhattan, Kansas, *Pres.;* J. J. Christensen, University of Minnesota, St. Paul, Minnesota, *Vice-Pres.;* M. B. Linn, University of Illinois, Urbana, Illinois, *Sec.-Treas.*

Pacific: S. M. Dietz, Oregon State College, Corvallis, Oregon, *Pres.;* William Newton, Plant Pathology Laboratory, Saanichton, British Columbia, *Vice-Pres.;* Roy A. Young, Oregon State College, Corvallis, Oregon, *Sec.-Treas.*

Potomac: H. Rex Thomas, Plant Industry Station, Beltsville, Maryland, *Pres.;* J. G. Leach. West Virginia University, Morgantown, West Virginia, *Vice-Pres.;* L. O. Weaver, University of Maryland, College Park, Maryland, *Sec.-Treas.*

Southern: T. W. Graham, Pee Dee Experiment Station, Florence, South Carolina, *Pres.;* J. A. Lyle, Alabama Agricultural Experiment Station, Auburn, Alabama, *Vice-Pres.;* G. B. Lucas, Plant Pathology, North Carolina State College, Raleigh, North Carolina, *Sec.-Treas.*

## SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Virginia.

The Dow Chemical Company, Midland, Michigan

E. I. du Pont de Nemours & Company, Incorporated, Wilmington 98, Delaware

Ferry-Morse Seed Company, P. O. Box 778, Detroit 31, Michigan

Freeport Sulphur Company, New Orleans 5, Louisiana

H. J. Heinz Company, Pittsburgh 30, Pennsylvania

Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Mathieson Chemical Corporation, Mathieson Building Baltimore, Maryland

Rohm & Haas Company, Washington Square, Philadelphia 5, Pennsylvania

Shell Chemical Corporation, Julius Hyman & Company Division, P. O. Box 2171, Denver 1, Colorado

Tennessee Corporation, 619-27 Grant Building, Atlanta 1, Georgia

Yoder Brothers, Inc., Barberton, Ohio

# HETEROCARYOSIS AS A BASIS FOR VARIATION IN PUCCINIA GRAMINIS VAR. TRITICI [1]

R. R. Nelson, Roy D. Wilcoxson, and J. J. Christensen

SUMMARY

Pathological and cytological studies were made to determine whether heterocaryosis is a basis for variation in *Puccinia graminis* var. *tritici*. Urediospores of 2 or more biotypes from distinctly different races of *P. graminis* var. *tritici* were mixed manually and dusted on compatible and noncompatible varieties of wheat. The first generation of urediospores from the compatible variety was transferred to resistant varieties of wheat. One biotype, originating from a mixture of biotypes of races 38 and 56, was highly pathogenic on Khapli, a wheat hitherto highly resistant to all known North American races of the wheat stem rust fungus. After several uredial generations on Khapli, the virulent heterocaryon became unstable, as indicated by a gradual loss of virulence on Khapli. After 25 uredial generations, it produced only type-1 infections (resistant reaction) on Khapli. Single-uredium isolations were made from type-1 infections on Khapli resulting from the dissociation of the virulent biotype. By this method, both parental biotypes were recovered and, in addition, 2 new races were obtained, each possessing some pathogenic characteristics of both parental biotypes.

Some of the urediospores, pedicels, and hyphal cells of the virulent biotype were 3- or 4-nucleate. The percentage of multinucleate cells and spores varied among isolates of the heterocaryon after 15 uredial generations, and the percentage also varied within a single isolate from one generation to another. The original biotypes and the new biotypes had only binucleate urediospores and did not dissociate.

It has been known for many years that new races and biotypes of parasitic fungi arise through hybridization, mutation and heterocaryosis. During the last 25 years it has been shown repeatedly that new biotypes of *Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Guyot are produced commonly by hybridization between existing biotypes and occasionally by mutation (11, 12, 19). Furthermore, it has been suggested that new biotypes of the stem rust organism may arise by means of vegetative fusion of dicaryotic hyphae and subsequent exchange and regrouping of nuclei (9). Recently Nelson and Wilcoxson (10) presented evidence that new races of *P. graminis* var. *tritici* arise by this method.

The experimental approach to the study of the nature and mechanisms of heterocaryosis in microorganisms has been used extensively only during the last 2 decades. The association, separation, and subsequent regrouping of different nuclei in multinucleate cells, as a result of hyphal fusions, have been firmly established as a mechanism of variation in many groups of fungi (2, 5, 7, 15).

The binucleate condition of mycelial cells and spores in a typical long-cycle rust organism, such as *P. graminis* var. *tritici*, has been established definitely by several workers (18). Variations in the number of nuclei, however, have been reported in nearly all stages of the life cycle of rust fungi. Allen (1), working with *Puccinia rubigo-vera* (DC.) Wint. var. *tritici* (Eriks. & E. Henn.) Arth., observed that the cells of the first 2 or 3 mycelial branches arising from the substomatal vesicle were 4- or 5-nucleate, and the cells of the subsequent hyphal branches were predominantly trinucleate. Multinucleate cells in the first hypha of the uredinal mycelium of *P. rubigo-vera* var. *tritici* were observed by Evans in 1907 (14). He observed them at a similar stage also in several other species of rusts. Some workers (6, 13) have observed that at the point of transition from the uninucleate condition to the binucleate, 3 nuclei have become associated instead of 2. Spores arising from these trinucleate cells also were trinucleate.

Hyphal fusions, prerequisite to nuclear exchange, also have been observed at various stages in the life cycles of rust species. Fusion of haploid hyphae is 1 means by which the dicaryophase is initiated at the base of the aecium (4). Brown (3), working with the autoecius rust fungus *Puccinia helianthi* Schw., showed that a dicaryotic mycelium may fuse with a haploid mycelium and give rise to the binucleate condition at the base of the aecium. Fusion between 2 dicaryotic hyphae has been observed in *P. graminis* var. *tritici* (17) and *P. rubigo-vera* var. *tritici* (1), respectively.

The literature on the cytology of rust fungi indicates that the binucleate condition in the reproductive area (sorus and associated hyphal cells) is stable. This phenomenon is of interest, since in many species of rust fungi a multinucleate condition is present in vegetative cells outside of the reproductive area. The mechanism or phenomenon responsible for the reestablishment of the binucleate condition is not known.

The preceding review indicates that fusion of vegetative hyphae and variations in the number of nuclei in cells occur in species of rust fungi. The purpose of the present research was to determine, by pathological and cytological methods, whether such variations can result in the production of heterocaryotic biotypes of *P. graminis* var. *tritici*. Heterocaryosis, as used in this text, means the association of more than 2 geneti-

[1] Accepted for publication July 11, 1955.
Paper No. 3326, Scientific Journal Series, Minnesota Agricultural Experiment Station.
Supported in part by the Rockefeller Foundation.

PHYTOPATHOLOGY for November (45: 581-638) was issued November 9, 1955

cally different kinds of nuclei in a vegetative cell or spore.

METHODS AND MATERIALS.—Attempts were made to demonstrate exchange of nuclei between different biotypes through the production of new biotypes with increased virulence and/or change in urediospore color. The same basic methods were used in both studies. Urediospores of 2 or more biotypes from distinctly different races of *P. graminis* var. *tritici* were mixed manually in approximately equal amounts and then diluted with talcum powder in a 1:5 ratio. The inoculum was dusted on compatible and noncompatible varieties of wheat with a cyclone duster. The term compatible designates a variety of wheat susceptible to all of the races in a mixture; noncompatible refers to a variety of wheat resistant to 1 or more of the races used. Host compatibility was considered a factor which might influence the success and/or degree of hyphal fusions.

Khapli emmer, highly resistant to all North American races of the wheat stem rust fungus, was used as a noncompatible wheat to determine whether virulent biotypes had been produced as a result of nuclear exchange. Since Khapli was resistant to all races cultured in the greenhouse, the possibility of contamination by virulent races or biotypes of races was eliminated. Little Club, susceptible to all of the races used in these studies, was used as the compatible variety.

Khapli and Little Club were inoculated with each mixture of races. Increased virulence on Khapli was detected directly by the results of the initial inoculations on Khapli. The first generation of urediospores produced on Little Club was transferred to Khapli and the resulting infections observed for the production of susceptible reactions on Khapli.

The purity of each of the races used in a mixture was confirmed repeatedly on the differential varieties of wheat used in the identification of races of the wheat stem rust fungus (20). Urediospores of each race to be included in a mixture were collected from plants of Little Club that were inoculated together with the other differential varieties.

The 24 races and biotypes of *P. graminis* var. *tritici* and the seed of the 21 varieties and selections of wheat used in the present studies were obtained from the Federal Rust Laboratory, St. Paul, Minnesota. The same isolate of each biotype was used in all of the studies.

One hundred fourteen mixtures of urediospores were studied on nearly 2 million wheat seedlings. Each mixture included urediospores of from 2 to 7 races or biotypes of the stem rust fungus. Most of the mixtures of urediospores were repeated, often several times. The mixture of urediospores of races 38 and 56 was repeated 48 times, and the next most frequent mixture was repeated 12 times.

EXPERIMENTAL RESULTS.—*Fusion of germ tubes.*—Although it is known that fusions occur between dicaryotic hyphae of the same biotype of *P. graminis* var. *tritici* (17), there is no evidence to show that similar fusions occur between hyphae of different biotypes. For that reason, normal-colored (red brown) urediospores of races 15B and 56, orange urediospores of a mutant of race 11, and gray-brown urediospores of a biotype of race 121 were mixed on a thin film of water agar, and their growth was observed under the phase microscope. Although differences in urediospore color are not discernible with a compound microscope, they are distinguished easily by means of a phase microscope.

Numerous hyphal fusions were observed 12–18 hours after germination. Hyphae were traced from the points of fusion to the spores from which they originated. Frequently, fusions involved hyphae from spores of different colors and consequently of different races.

With the knowledge that fusions occur frequently between hyphae of certain races of *P. graminis* var. *tritici*, studies were made in the greenhouse to determine if exchange of nuclei occurs between different biotypes in the dicaryophase and if genetic variations might result from new associations of nuclei.

*Pathological studies.*—As a result of inoculations with mixtures of urediospores of different biotypes, 4 new biotypes were produced that differed from the original biotypes in virulence and/or color. One biotype originating from a mixture of biotypes of races 38 and 56 was highly pathogenic on Khapli emmer. In the present paper, which presents the results of the studies with the mixture of biotypes of races 38 and 56, the biotype virulent on Khapli will be referred to as the Khapli heterocaryon. Approximately 1 million wheat seedlings were inoculated with 48 urediospore mixtures of biotypes of 38 and 56. The Khapli heterocaryon was produced twice from this mixture, once in the winter of 1953 and again in the late summer of 1954.

The Khapli heterocaryon was more virulent than

TABLE 1.—*Reaction of different varieties of Triticum spp. to old and new races of P. graminis var. tritici*

| Differential varieties | Varietal reaction to[a] | | | | |
|---|---|---|---|---|---|
| | 38[b] | 56[b] | Hetero-caryon | Race A[c] | Race B[c] |
| Marquis | R | S | S | S | S |
| Reliance | S | S | S | S | S |
| Kota | S | S | S | S | S |
| Arnautka | X | R | S | S | S |
| Spelmar | X | R | S | X | X |
| Mindum | X | R | S | X | X |
| Kubanka | X | S | S | X | S |
| Acme | X | S | S | S | X |
| Einkorn | S | R | S | S | R |
| Vernal | R | R | R | R | R |
| Khapli | R | R | S | R | R |

[a] R indicates variety was resistant; S indicates variety was susceptible; X signifies variety was mesothetic (uredia variable, sometimes including all infection types and intergradations between them on the same leaf).

[b] Reactions of original biotypes used in mixtures of urediospores as well as of biotypes derived from the dissociation of the heterocaryon.

[c] New race derived from dissociation of Khapli heterocaryon.

either of the original biotypes comprising the mixture. The increased virulence was evident not only on Khapli emmer (Fig. 1), but also on several other varieties of the differential series. The virulence of the heterocaryon exceeded that of both original biotypes on the varieties Arnautka, Mindum, and Spelmar (Table 1).

The reactions of Khapli to the Khapli heterocaryon and to the original biotypes of 38 and 56 were tested under a wide range of environmental conditions, ranging from low temperatures (18°–21°C) and low light intensity to high temperatures (27°–30°C) and supplementary light. Khapli was susceptible to the heterocaryon and resistant to the biotypes of 38 and 56 under all conditions tested.

After its production, the Khapli heterocaryon had a marked degree of instability as indicated by a gradual loss of virulence on Khapli. After 25 uredial generations, it produced only type-1 infections (resistant reaction). With each succeeding uredial generation, a greater percentage of the reactions of Khapli were of the resistant type. The reaction of Khapli to the heterocaryon after 8 uredial generations is shown in Figure 1. At that time, susceptible reactions were most prevalent, but occasionally scattered resistant reactions occurred.

Twenty-five single-uredium isolates, obtained from 25 type-4 infections (susceptible reaction) on Khapli, were studied from the tenth to the fifteenth uredial generations to observe the rate and degree of dissociation of the heterocaryon on Khapli. Certain isolates produced predominently type-4 infections and a few type-1 infections on Khapli; other isolates produced nearly equal numbers of type-4 and type-1 infections on Khapli; and still other isolates produced predominantly type-1 infections on Khapli. During the studies, no isolate from type-4 infections produced only type-1 infections on Khapli.

After 15 uredial generations, the reactions of Khapli when inoculated with any of the isolates of the Khapli heterocaryon were predominantly of the resistant type. Isolations were made from the type-1 infections on Khapli, and each such isolate was increased separately on Little Club wheat and then identified on the

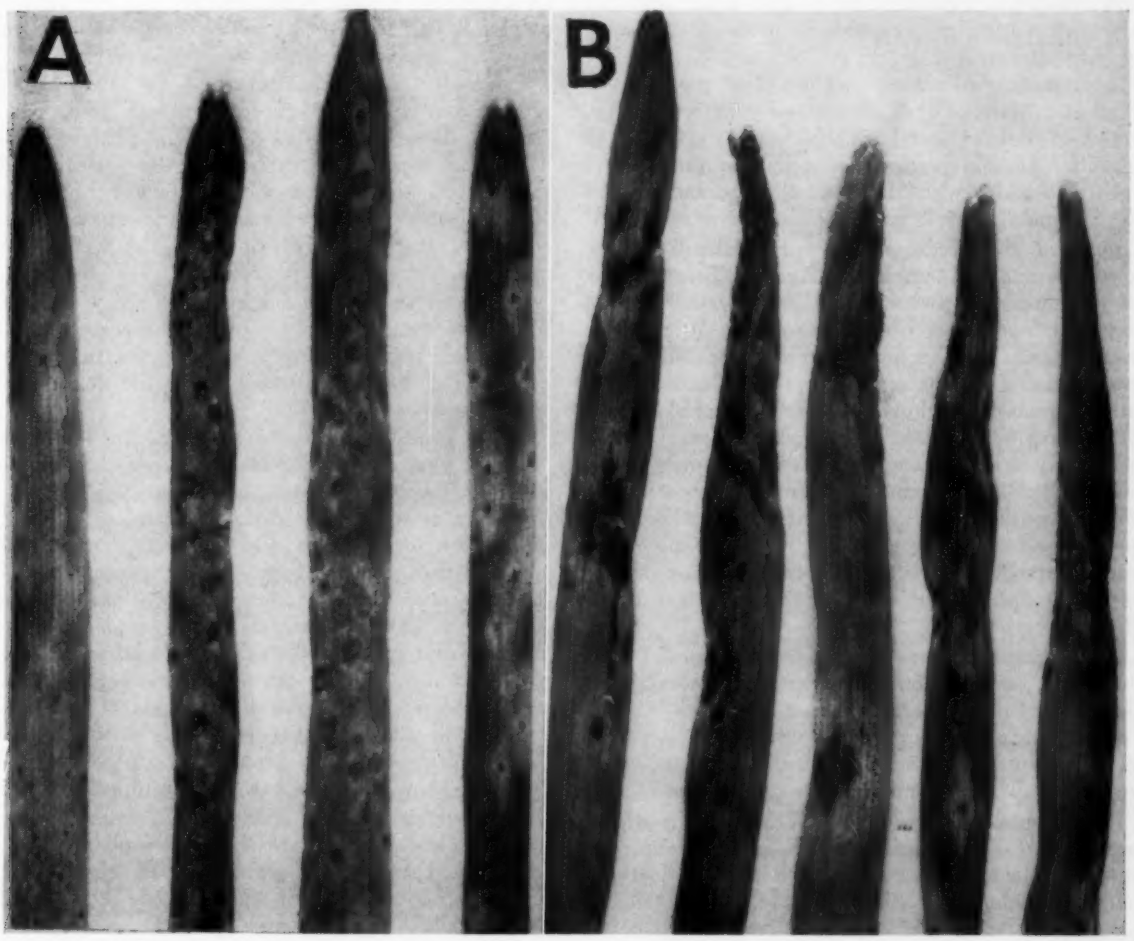



FIG. 1. Reaction of Khapli emmer to races 38 and 56 and to the Khapli heterocaryon (38 + 56) of *P. graminis* var. *tritici*. A) Left, leaf inoculated with race 56; middle, 2 leaves inoculated with Khapli heterocaryon; right, leaf inoculated with race 38. B) Reaction of Khapli to the Khapli heterocaryon after 8 uredial generations on Khapli.

differential varieties. Some of the isolates identified were race 38 and some were race 56, the original races used in the mixture from which the heterocaryon was derived. In addition, at least 2 new races, A and B, dissociated from the heterocaryon, were isolated from the type-1 infections on Khapli. Each new race possessed some pathogenic qualities of the original races, 38 and 56. The variety Marquis was as susceptible to both new races as to race 56, and the varieties Mindum and Spelmar had the mesothetic reaction that they usually have to race 38. New race A differed from new race B on Kubanka, Acme, and Einkorn. Race A and B were more virulent on Arnautka than either races 38 or 56 (Table 1). Neither race A nor race B are identical to any known physiologic race of *P. graminis* var. *tritici*.

*Cytological studies.*—Studies were made to determine the nuclear condition of the original biotypes of 38 and 56, and the Khapli heterocaryon. Sections of leaf tissue, approximately 5 mm square and containing 1 or more rust pustules, were used. Pustules representing different stages of development of the rust were examined. Satisfactory stains were obtained only with mature urediospores in pustules that were not yet erumpent.

In preparing microtome sections, leaf tissues were killed and fixed in weak chrom-acetic solution, dehydrated in normal butyl alcohol, and embedded in paraffin. Paraffin sections were cut 6$\mu$ thick, fixed serially to slides with Haupt's adhesive, and stained with Flemming's triple stain (8).

Some of the urediospores of the Khapli heterocaryon were 3- or 4-nucleate (Fig. 2). Furthermore, the percentage of urediospores with 3–4 nuclei varied from less than 1 per cent to more than 10 per cent among uredia of the heterocaryon after 20 uredial generations. Such variation appeared to be associated with differences in pathogenicity among uredial isolates. Preliminary observations of urediospores representing the initial uredial generations of the heterocaryon indicate that the percentage of urediospores with 3–4 nuclei is considerably higher in early generations. This appears to be closely associated with the more consistent pathogenicity of the heterocaryon on Khapli during the first generations after the production of the Khapli heterocaryon.

Urediospores of the biotypes of 38 and 56 were binucleate, a condition considered as normal for urediospores of the stem rust fungus. Figure 2 shows a section through a uredial sorus of the biotype of race 56, one of the original races. The section is typical of all sori examined for races 38 and 56.

Urediospores of new races A and B, produced through the dissociation of the Khapli heterocaryon, were binucleate. The production of 2 new biotypes, both dicaryons, from a heterocaryon indicates that a reassortment of nuclei within a heterocaryotic cell resulted in the production of a dicaryon with 1 nucleus from each of the 2 original races comprising the mixture.

Studies also were made on the nuclear constitution of pedicels and vegetative hyphal cells in the reproductive areas of the Khapli heterocaryon and the biotypes of races 38 and 56. Vegetative hyphal cells were stained with the modified quadruple stain (8) and examined for nuclear condition. Ramification of the hyphae was observed best in sections 8$\mu$ and 10$\mu$ thick.

Pedicels and hyphal cells of the original biotypes of 38 and 56 were binucleate. Hyphal cells were observed throughout the reproductive areas and often well beyond the limits of the areas. All of the hyphal cells observed were binucleate.

Usually, pedicels bearing trinucleate urediospores of the Khapli heterocaryon were trinucleate also. Furthermore, hyphal cells in the reproductive areas often were trinucleate, but exceptions to the trinucleate condition were noted. Occasionally, urediospores with 3–4 nuclei were borne on pedicels with 2 nuclei and, conversely, trinucleate pedicels were associated with binucleate urediospores in some cases. Similar variations were observed in hyphal cells in the host tissues. Occasionally, hyphae with trinucleate cells branched and gave rise to 1 hypha with trinucleate cells and a second hypha with binucleate cells.

Cytological investigations also were made of sori produced as a result of inoculation with urediospores from a single pustule of the Khapli heterocaryon. There was considerable variation in the nuclear condition among sori. Some were uniformly binucleate, whereas others contained many trinucleate cells. No sori were observed in which all of the urediospores and stalk cells were trinucleate.

Such variation in the number of nuclei within cells of the heterocaryon suggests a random segregation of nuclei that begins at any point from the germination of the heterocaryotic urediospore to the development of the new sorus.

Discussion.—The results presented in this paper indicate that hyphal fusions between biotypes of races of *P. graminis* var. *tritici* and subsequent nuclear exchange or new associations of nuclei result in the production of heterocaryotic biotypes that are more virulent than the biotypes of the "parental" races. The new and often temporary associations of nuclei apparently provide another means, aside from the usual sexual process, of "bringing together" different genes, as measured by the total genic action. In the case of heterocaryosis, the different genes are brought together in the sense that genetically different nuclei become grouped within a multinucleate cell.

The evidence indicates that a multinucleate condition in urediospores is unstable. The time required for complete reversion to the dicaryophase may be conditioned by chance segregation of nuclei, which may occur at any point in the life cycle of the uredial stage.

The fact that the Khapli heterocaryon was produced on 2 occasions from a mixture of urediospores of the same isolates of biotypes of 38 and 56 greatly

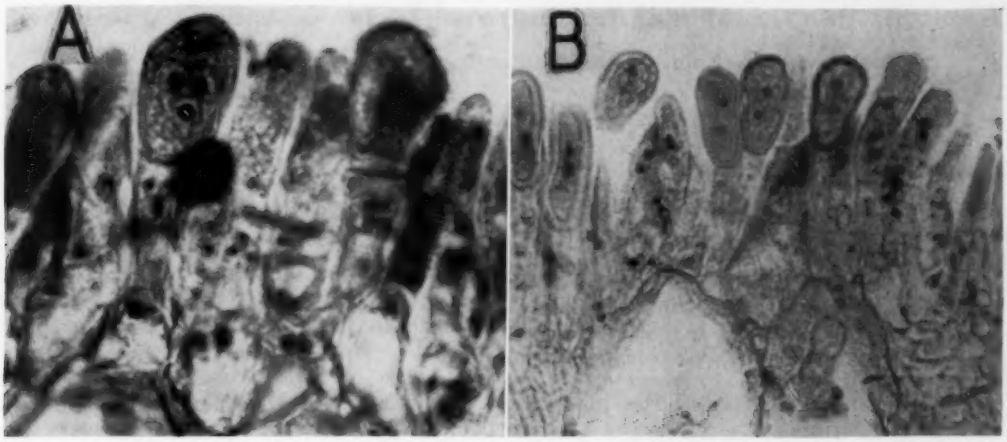



FIG. 2. Nuclear condition of urediospores of races of *P. graminis* var. *tritici*. A) Urediospore of Khapli heterocaryon with 3 nuclei. (Photomicrograph, ×1400). B) Urediospores of race 56 with 2 nuclei. (Photomicrograph, ×900).

minimizes the possibility that gene mutation could account for the increased pathogenicity of the heterocaryon.

It is highly probable that heterocaryotic biotypes are produced in nature. Extensive acreages of wheat, planted with a variety or varieties susceptible to more than 1 biotype or race of stem rust, and the billions of urediospores produced in a single acre of wheat afford an excellent opportunity for heterocaryotic biotypes to be produced. Furthermore, when such heterocaryotic biotypes are cultured extensively during greenhouse studies, it is likely that biotypes will be obtained that differ from the original heterocaryon, as a result of a reassociation of nuclei. In some cases it would be difficult or perhaps impossible to determine whether a mutation for pathogencity had occurred (12) or a new heterocaryon had originated.

The results of the present studies demonstrate that the genes for virulence on Khapli are present in North American races of *P. graminis* var. *tritici* and can be associated in different nuclei within a cell as a result of hyphal fusions. Such knowledge would be of great practical importance in evaluating the total genic potential of *P. graminis* var. *tritici*.

The phenomenon of heterocaryosis may be useful as a potential tool for the examination of the genic action of rust pathogens and also may be useful in the study of rust fungi whose sexual phase is not common, but in which there is considerable diversity of races.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
UNIVERSITY OF MINNESOTA
INSTITUTE OF AGRICULTURE
ST. PAUL 1, MINNESOTA

LITERATURE CITED

1. ALLEN, RUTH F. 1926. A cytological study of Puccinia triticina physiologic form 11 on Little Club wheat. Jour. Agr. Res. **33**: 201-222.
2. BEADLE, G. W., AND V. L. COONRADT. 1944. Heterocaryosis in Neurospora crassa. Genetics **29**: 291-308.
3. BROWN, A. M. 1932. Diploidization of haploid by diploid mycelium of Puccinia helianthi Schw. Nature [London] **130**: 777.
4. CRAIGIE, J. H. 1927. Experiments on sex in rust fungi. Nature [London] **120**: 116-117.
5. DODGE, B. O. 1942. Heterocaryotic vigor in Neurospora. Bul. Torrey Bot. Club **69**: 75-91.
6. FROMME, F. D. 1914. The morphology and cytology of the aecidium cup. Bot. Gaz. **58**: 1-35.
7. HANSEN, H. N. 1938. The dual phenomenon in imperfect fungi. Mycologia **30**: 442-455.
8. JOHANSEN, D. A. 1940. Plant microtechnique. Ed. 1. McGraw-Hill, New York. 523 p.
9. JOHNSON, T. 1953. Variation in the rusts of cereals. Biol. Rev. **28**: 105-157.
10. NELSON, R., R., AND R. D. WILCOXSON. 1954. Heterocaryosis as a mechanism of variation in rust fungi. (Abs.) Phytopathology **44**: 500.
11. NEWTON, M., AND T. JOHNSON. 1932. Studies in cereal diseases. VIII. Specialization and hybridization of wheat stem rust, Puccinia graminis tritici, in Canada. Canad. Dept. Agr. Bul. 160, n.s. 60 p.
12. NEWTON, M., AND T. JOHNSON. 1939. A mutation for pathogencity in Puccinia graminis tritici. Canad. Jour. Res. Sect. C, **17**: 297-299.
13. OLIVE, E. W. 1908. Sexual cell fusions and vegetative nuclear divisions in the rusts. Ann. Bot. [London] **22**: 331-360.
14. POLE EVANS, I. B. 1907. The cereal rusts. I. The development of their uredo mycelia. Ann. Bot. [London] **21**: 441-466.
15. PONTECORVO, G., AND A. R. GEMMELL. 1944. Genetic proof of heterocaryosis in Penicillium notatum. Nature [London] **154**: 514.
16. RAPER, J. R., AND J. P. SAN ANTONIO. 1954. Heterokaryotic mutagenesis in Hymenomycetes. I. Heterokaryosis in Schizophyllum commune. Amer. Jour. Bot. **41**: 69-86.
17. RODENHISER, H. A., AND ANNIE M. HURD-KARRER. 1947. Evidence of fusion bodies from urediospore germ tubes of cereal rusts on nutrient solution agar. Phytopathology **37**: 744-756.
18. SAVILE, D. B. O. 1939. Nuclear structure and behavior in species of the Uredinales. Amer. Jour. Bot. **26**: 585-609.
19. STAKMAN, E. C., M. N. LEVINE, AND R. U. COTTER. 1930. Origin of physiologic forms of Puccinia graminis through hybridization and mutation. Sci. Agr. **10**: 707-720.
20. STAKMAN, E. C., M. N. LEVINE, AND W. Q. LOEGERING. 1944. Identification of physiologic races of Puccinia graminis tritici. U.S. Dept. Agr. Bur. Ent. and Pl. Quarant. Bul. 617, 27 p.

# TEMPERATURE AND THE GEOGRAPHICAL DISTRIBUTION OF CHRYSANTHEMUM RUST [1]

Colin E. Campbell and A. W. Dimock [2]

SUMMARY

The optimum temperature range for germination of urediospores of *Puccinia chrysanthemi* is from 60° to 70°F (15-21°C). Percentage of germination drops very rapidly on either side of this optimum range, and no germination occurs in 24 hours at 43° or 87°F (6° or 30°C). Leaf infection occurs between 60 and 81°F (15° and 27°C), but does not occur at 43° or at 87°F. In infections up to 12 days old, the pathogen was killed by exposure to air temperatures of 87°F for 24 hours or 101°F (38°C) for only 8 hours. In incipient infections up to 48 hours old, the pathogen was killed by 4 hours exposure to 101°F. Spores in uredia were killed by 4 daily 4-hour exposures to 101°F, whereas germination was reduced almost 90 per cent by 3 such exposures to 101°F or 5 exposures to 87°F. Leaf temperature in direct sunlight in the open was found to exceed the ambient temperature by as much as 16°F (9°C). Thus, in insolated leaves the pathogen might be eradicated at air temperatures favorable for continued rust development. The thesis that high temperature is a decisive factor determining the geographical distribution of chrysanthemum rust in North America is supported by these studies.

Chrysanthemum rust, caused by *Puccinia chrysanthemi* Roze, has been reported from most of the areas in which the common cultivated chrysanthemum, *Chrysanthemum morifolium* Ramsb. (*C. indicum* L.), is grown. In North America, however, it becomes serious only in the cooler regions along the Pacific coast and, sporadically, along the northern Atlantic coast. Although the fungus unquestionably is introduced repeatedly into inland areas, it fails to become established and to cause important damage, except occasionally in winter months in greenhouses. In exploratory studies at Ithaca, it was found extremely difficult to establish infection in experimental greenhouses during summer months, although heavy infection was obtained readily in cooler months when the same inoculation and incubation techniques were employed. Even when the plants were incubated in low-temperature chambers for a brief period after inoculation, it still was difficult to establish the disease in summer. Furthermore, on plants that already were infected, the rust fungus appeared to die out or at least to degenerate in its spore-producing ability and destructiveness. These observations suggested that temperature is one of the more critical environmental factors affecting chrysanthemum rust development. This was substantiated by the studies reported here.

EXPERIMENTAL STUDIES.—*Temperature and spore germination.*—1) *Methods and materials.*—Freshly-collected spores were dusted on agar surfaces in Syracuse watch glasses that had been preadjusted to the test temperatures, and germination counts were made 24 hours after the preparations had been returned to the controlled-temperature chambers. In preliminary tests, germination on 2 per cent water agar in Syracuse watch glasses did not differ significantly from that in water on slides, as described by others (5, 7); germination usually was 70–95 per cent. The use of agar in Syracuse watch glasses had these advantages: the spores did not clump together as they did in water drops, and hence were much easier to count reliably; the watch glasses could be stacked and thus refrigerator space could be saved; and the heavy watch glasses changed little in temperature when removed briefly from the temperature boxes to sow the spores. Preliminary tests had shown that stacking the watch glasses did not reduce germination.

Preadjusting the medium to the test temperature, as noted by Cochrane in studies on the rose rust fungus (1), was necessary to avoid erroneous results. In a preliminary trial, 1 set of watch glasses containing 2 per cent water agar was precooled to the germination temperatures for 24 hours, while another set was kept at room temperature before the agar was sown with urediospores. All were then sowed and placed immediately at the test temperatures. After 24 hours, counts showed 13, 67, and 90 per cent germination at 44°, 49°, and 54°F, respectively, for the non-precooled series, whereas the figures for the precooled series were 0, 2, and 8 per cent, respectively. Urediospores of *P. chrysanthemi* and many other rust fungi germinate in 2–4 hours, but the change in temperature of Syracuse watch glasses may be relatively slow; hence, unless temperatures of equipment and germination surfaces are preadjusted, appreciable germination may occur before the test temperatures are reached.

Spores for the tests were composite collections from leaves bearing uredia of various ages, since tests had failed to indicate any need for standardization as reported by Cochrane (1) for the rose rust fungus. Following collection, spores were diluted with talc and dusted on the agar with a small puff duster. A germination period of 24 hours was adopted for convenience.

[1] Accepted for publication July 21, 1955.

[2] Technical representative, Velsicol Chemical Corporation, Chicago, Illinois, and Professor of Plant Pathology, Cornell University, Ithaca, New York, respectively.

Preliminary trials had shown that at 65°F no appreciable additional germination occurs after the first 12 hours. In each test there were 4 replicates for each temperature.

2) *Results.*—Results of 2 independent tests (Fig. 1) are in substantial agreement with those of Gibson (4), who observed no germination in 24 hours at 43° or 87°F and a reduction in germination above 70°. Although there is a rather flat optimum range (approximately 60–70°F) for maximum germination, the drop on either side seems surprisingly abrupt. This is not inconsistent, however, with the apparent sensitivity of the disease to temperature conditions in field and greenhouse. It is the writers' opinion that the less abrupt temperature curves often shown for spore germination may in some cases reflect failure to adjust the germination medium to the test temperature before the spores are sown. Not shown by the germination figures are the facts that at the higher temperatures the germ tubes not only were decidedly shorter but were more contorted than at lower temperatures and the walls were crenulate, as observed previously by Gibson.

*Temperature and rust development in excised leaves.*—In these tests, leaves were floated, lower surface up, on 7 per cent sucrose solution in covered petri plates after the method described by Yarwood (8). The leaves were dusted with a talc-urediospore mixture and sprayed with tap water. The petri plates then were placed at the desired temperatures.

A test was conducted in which inoculated leaves were placed immediately at 43°, 60°, 70°, 81°, and 87°F and allowed to remain undisturbed. Three replicates were placed at each temperature. At the end of 3 weeks, no rust had developed at 43° or 87°, but all 3 replicates at 60° showed rust, as did 2 of 3 at 70° and 1 of 3 at 81°.

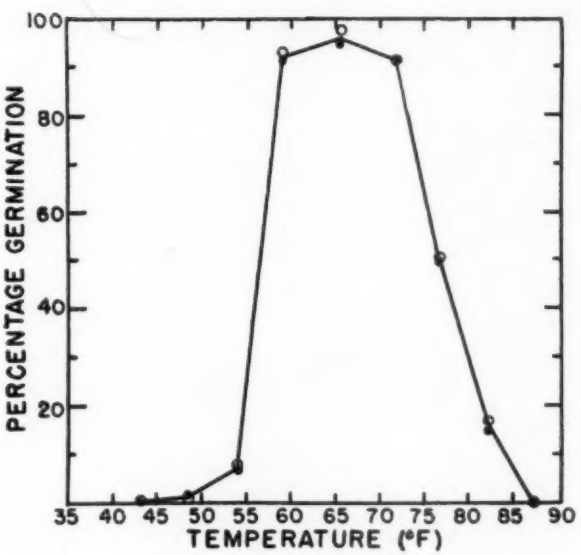



FIG. 1. Percentage germination of urediospores of *Puccinia chrysanthemi* in 24 hours at different temperatures. Graph represents average of 2 tests; actual points of the 2 tests are indicated by open circles and by closed circles.

A second test was conducted in which the leaves remained at the optimum temperature of 65°F for 24 hours after inoculation, then were placed at 101° for ½, 1, 2, 4, 8, and 24 hours, and finally were returned to room temperature. Three replicates were used at each temperature. Rust developed on all leaves exposed to 101° for 2 hours or less, but not on leaves that had been exposed for 4 hours or more. It should be recognized that the duration of exposure to 101° was actually less than that indicated, since considerable time necessarily elapsed before the petri dishes and leaves, starting at 65°, reached the test temperature; possibly those in the ½-, 1-, and 2-hour series did not reach 101°.

*High temperature exposure of entire plants.*—1) *Test 1.*—a) *Procedure.*—Five leaves on each of 14 plants of the variety Yellow Fellow were inoculated with urediospore-talc dust and sprayed with tap water. The plants were then placed in an improvised greenhouse moist chamber in which the temperature ranged from 60° to 72°F. After 24 hours, 8 plants were removed from the moist chamber. Two of these were placed on the open greenhouse bench to serve as checks, and the other 6 were placed in an incubator at approximately 101°. After 2, 4, and 24 hours, 2 plants were removed from the incubator and placed on the greenhouse bench. The remaining 6 plants were removed from the greenhouse moist chamber after 48 hours; 2 of these were placed immediately on the greenhouse bench, 4 were placed in the 101° incubator where 2 were left for 2 hours and 2 for 4 hours before being returned to the greenhouse bench. The check plants were heavily infected after 2 weeks, but to eliminate the possibility that rust development on treated plants was merely retarded by the high temperature treatments, records were not taken until 4 weeks had elapsed.

b) *Results.*—The results (Table 1 and Fig. 2) show that exposure of recently inoculated plants to 101°F for as little as 4 hours will completely inhibit establishment of infection. In view of the incubation period of 48 hours initially given some of the plants, it is indicated that in recently established infections the pathogen actually is eradicated within the host tissue by brief exposure to high temperature.

TABLE 1.—*Average number of primary rust pustules per inoculated leaf on chrysanthemums following brief exposures to 101°F subsequent to inoculation and incubation at 60–72°F*

| Hours at 101°F. | Pustules, when incubation period at 60–72°F had been | |
|---|---|---|
| | 24 hours | 48 hours |
| 0 | 300–500 | 300–500 |
| 2 | 5 | |
| 4 | 0 | 0 |
| 24 | 0 | 0 |

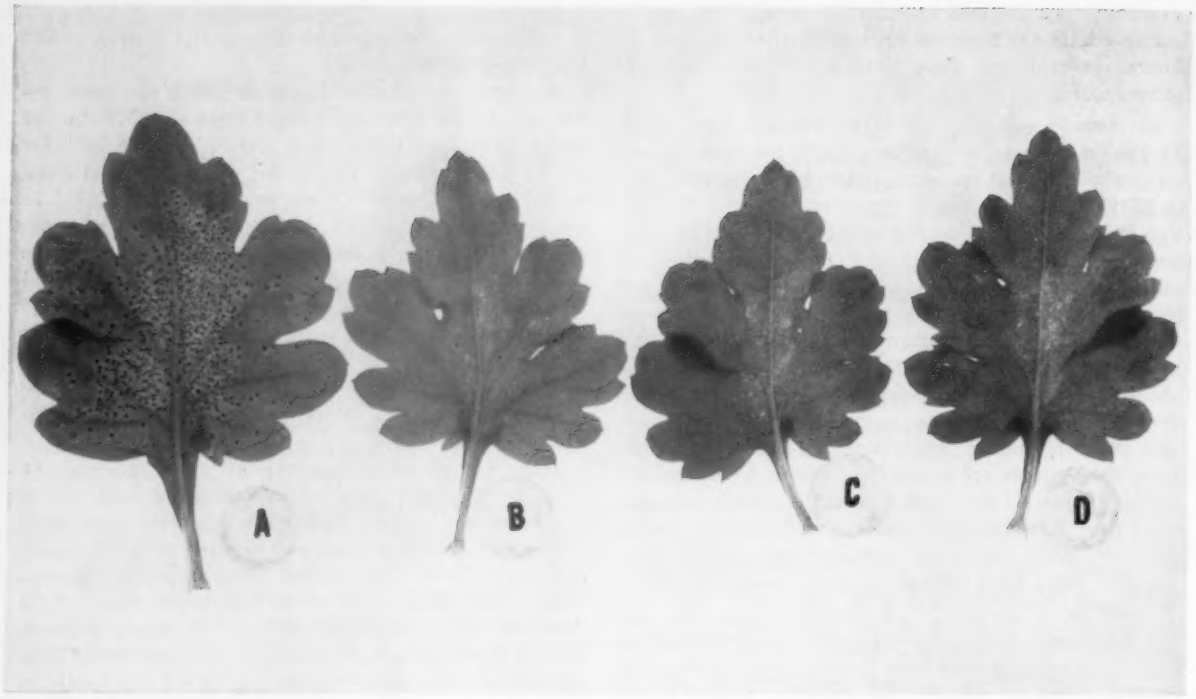



FIG. 2. Leaves from chrysanthemum plants that had been inoculated with urediospore-talc mixture, placed in greenhouse moist chamber at 60–72°F for 24 hours, then exposed to 101°F for different periods before being placed on the greenhouse bench. A) No 101° treatment. B) 101° for 2 hours. C) 101° for 4 hours. D) 101° for 24 hours. White deposit on leaves is talc.

2) *Test* 2.—a) *Procedure.*—In another experiment, plants were subjected to high temperature after they had been incubated at favorable temperatures for 7 and 12 days, the uredial flecks first being visible at the end of the latter period. Three leaves on each of 39 plants were inoculated as described before, placed in the improvised greenhouse moist chamber for 24 hours, and then placed on the greenhouse bench. The temperature did not exceed 76°F during the first 3 days, reached a maximum of 88° for a very short period the fourth day, and reached 103° very briefly the sixth day.

Seven days after initiation of the test, 9 inoculated plants were placed in an incubator at 87°F and 9 in an incubator at 101°. After 4-, 8-, and 24-hour periods, 3 plants from each incubator were returned to the greenhouse bench. Twelve days from the start of the tests, when the first uredial flecks were evident, the same procedure was followed with 18 other inoculated plants. The remaining 3 were left on the greenhouse bench as checks.

b) *Results.*—The differential effect of temperature on both infected and healthy leaf tissues was striking (Table 2). Healthy tissue was uninjured except by the 24-hour exposure to 101°F; here there was marginal burning of the upper leaves. Of the other plants, those which were incubated only 7 days at favorable temperatures prior to high temperature exposure showed no injury. The most interesting effects were shown by the plants that were incubated 12 days at favorable temperatures before high temperature treatment. On the plants exposed only 4 hours to 101°, there was selective killing of the leaf tissue coincident with the uredial flecks that had appeared prior to treatment. The tissue of healthy leaves, and even of infected leaves in areas other than where the flecks occurred, was uninjured. Plants exposed to 101° for 8 hours after a 12-day incubation period also showed

TABLE 2.—*Rust pustule development on chrysanthemum leaves inoculated and incubated at favorable temperatures for 7 and 12 days, then exposed to high temperatures for different time intervals*

| Days at favorable temperature | Hours at high temperature | Pustules per leaf[a] following exposure at indicated high temperature | |
|---|---|---|---|
| | | 87°F | 101°F |
| 7 | 0 | 200[b] | |
| | 4 | 200[b] | 10 |
| | 8 | 200[b] | 0 |
| | 24 | 5 | 0[e] |
| 12 | 4 | 200[b] | 5[c] |
| | 8 | 200[b] | 0[d] |
| | 24 | 0[d] | 0[e] |

[a] Three leaves examined on each of 3 plants.
[b] Estimated; at least 200 pustules per leaf.
[c] Infected tissue killed where flecks were numerous.
[d] Infected tissue killed; noninfected tissue not injured.
[e] Infected tissue killed; noninfected tissue severely injured.

injury only on the infected leaves, though somewhat more severe than on the leaves exposed only 4 hours (Fig. 3).

Evidently, relatively brief exposures to high temperature may adversely affect rust development even though the disease is well established. An exposure of somewhat over 24 hours at 87°F may be needed for complete eradication of the pathogen, but at 101° as little as 8 hours is sufficient.

*High temperature and spore viability.*—To determine the cumulative effect of consecutive brief high-temperature exposures on the viability of urediospores, 3 plants with sporulating uredia were exposed to 4-hour periods at 87°F on 5 successive days. Three plants were similarly exposed to 4-hour periods at 101°. After each exposure period, spores from each group of plants were harvested, mixed, sown on 2 per cent water agar in Syracuse watch glasses, and placed at 65°F for 24 hours.

The germination figures for the 87°F series lack reliability, because of variation among replicates (Table 3), yet the trend is quite evident. It would appear that even at this temperature spore viability is greatly reduced, the effect of successive exposures being cumulative. Presumably, viability would have been wholly lost after 1 or 2 more 4-hour exposures. Gibson (4) found that harvested urediospores exposed to 87° in germination tests (presumably kept moist or at very high humidity for 24 hours) failed to germinate when returned to lower temperature; however, harvested spores stored dry at 96° for 18 hours germinated freely when returned to 63°.

FIG. 3. Effect of 8-hour exposure to 101°F on infected and noninfected chrysanthemum leaves. Three leaves indicated by arrows were inoculated 12 days prior to the 101°F treatment, and uredial pustules were present.

Exposure to 101° for as little as 4 hours resulted in drastic reduction of urediospore germination. Furthermore, in tests made after the plants had been returned to the greenhouse bench and kept for an additional 2 weeks, none of the spores collected from plants of the 101° series were viable. Evidently the fungus had been eradicated by even a 4-hour exposure to 101° in this test.

*Comparison of leaf and air temperatures.*—It has been reported by Stevens (6) and Curtis (2) that leaves in sunlight may attain temperatures considerably above those of the ambient air. To study the temperature of chrysanthemum leaves, copper-constantan thermocouples were threaded through the leaves as described by Curtis. Plants were placed in sunlight or in shade both in the greenhouse and out-of-doors, and temperatures were determined on the basis of galvanometer readings. Thermocouples were placed in the air adjacent to the test leaves to obtain comparable ambient air temperatures.

Evidently, in sunlight in the open the temperature of unshaded leaves may exceed the registered air temperature by at least 16°F (Table 4). Thus, the temperatures of 87° and 101° used in the preceding tests might be reached in unshaded leaves at registered air temperatures of only 71° and 85°, respectively.

DISCUSSION.—These studies have shown that high temperature—above 80°F—may be an important deterrent to the establishment of chrysanthemum rust in 3 ways: by reducing germination of initially viable urediospores at the infection court, by eradicating established infections, and by killing spores in existing uredia. Although other factors are involved, temperature may well be the most important one determining the geographical distribution of this disease. The

TABLE 3.—*Percentage germination of spores harvested from uredia on leaves of chrysanthemum plants that had been exposed to high temperatures for 4 hours daily on successive days*

| No. of 4-hour exposures | Percentage germination[a] following exposure to indicated temperature | |
|---|---|---|
| | 87°F | 101°F |
| 0[b] | 85.5 | 82.6 |
| 1 | 75.3 | 28.4 |
| 2 | 67.3[c] | 22.1 |
| 3 | 55.6[c] | 8.6 |
| 4 | 17.6[c] | 0 |
| 5 | 6.1[c] | 0 |

[a] Based on counts of 100 spores in each of 3 replicate plates.

[b] Check plants kept on greenhouse bench; temperature varied from 60 to 100°F, but exceeded 78°F on only a few days.

[c] Significant difference between replicates.

TABLE 4.—*Air and leaf temperatures determined by thermocouples*

| Thermocouple location | Location of plants: Unshaded[a] greenhouse °F[b] | Unshaded greenhouse: Departure from air | Out-of-doors °F[b] | Out-of-doors: Departure from air |
|---|---|---|---|---|
| Sun, Air | 88.0 | | 67.0 | |
| Sun, leaf | 92.4 | +4.4 | 83.0 | +16.0 |
| Shade, air | 85.2 | | 62.0 | |
| Shade, leaf | 82.8 | −2.4 | 63.0 | +1.0 |

[a] Glass clear, not painted with shading compound.
[b] Values are averages of 5 consecutive readings taken at 1-minute intervals.

North American areas where the disease is common and important are generally cool. In contrast, those areas where the disease is absent are characterized by hot summers, even though the factors of rain and dew may be favorable for infection. Cochrane (1), in his study of rose rust, showed that at San Diego, California, where both rose rust and chrysanthemum rust are serious, there were only 52 days in a 2-year period (1941-1942) when the temperature was 80° or higher, whereas at Ithaca, New York, where neither disease becomes established, there were 152 such days. Dimock and Baker (3), in analyzing weather records for the period July 16 to September 5, 1947, found that the high-temperature records for Los Angeles, California, and Ithaca, New York, differed only slightly, but the average daily temperature was much more frequently in the favorable range (60–70°F) for chrysanthemum rust at Los Angeles, where the disease is common, than at Ithaca. They found further that at Wooster, Ohio; Beltsville, Maryland; and Urbana, Illinois, locations where chrysanthemum rust is unimportant, the maximum and average daily temperatures greatly exceeded those at Los Angeles, often being in the range lethal to the pathogen in established infections.

Within greenhouses in most inland and southern areas, summer temperatures commonly are 90°F or higher for many hours each day over extended periods. Under such temperature conditions, chrysanthemum rust could not become established even though all other factors were favorable.

An effect of temperature on the rust distribution pattern on the individual plant is suggested by the leaf-temperature studies. It is commonly felt that the greater abundance of infections on the lower and inner leaves is due chiefly to the fact that these leaves dry off less rapidly after rain or dew. Conditions thus would be favorable for germination and infection a much longer time than on the upper and outer leaves. This no doubt is true, but it is suggested here that insolation and the consequent rise in leaf temperature also may affect the pattern by eradication of the pathogen in incipient infections initiated in exposed leaves during periods of lower temperature or cloudy weather.

Although low-temperature effects were not studied extensively, failure to obtain germination or infection at 43°F indicates that low temperatures during fall, winter, and spring in most inland areas and along the northern Atlantic coast also may be very important in restricting chrysanthemum rust.

DEPARTMENT OF PLANT PATHOLOGY
CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. COCHRAN, V. W. 1945. The common leaf rust of cultivated roses, caused by Phragmidium mucronatum (Fr). Schlect. N. Y. (Cornell) Agr. Exp. Sta. Mem. 268, 39 p.
2. CURTIS, O. F. 1936. Leaf temperatures and the cooling of leaves by radiation. Pl. Physiol. **11**: 343-364.
3. DIMOCK, A. W., AND K. F. BAKER. 1951. Effect of climate on disease development, injuriousness, and fungicidal control, as exemplified by snapdragon rust. Phytopathology **41**: 536-552.
4. GIBSON, C. M. 1904. Notes on infection experiments with various Uredineae. New Phytol. **3**: 184-191.
5. LOEGERING, W. Q. 1941. A satisfactory medium for germination of urediospores of Puccinia graminis tritici. Phytopathology **31**: 952-953.
6. STEVENS, N. E. 1921. Environmental temperatures of fungi in nature. Amer. Jour. Bot. **9**: 385-390.
7. STRAIB, W. 1940. Phytologische Untersuchungen über Puccinia glumarum. Zentbl. f. Bakt. [etc.] Abt. II, **103**: 154-188.
8. YARWOOD, C. E. 1934. The comparative behavior of four clover-leaf parasites on excised leaves. Phytopathology **24**: 797-806.

# RELATION OF SOFT ROT DEVELOPMENT TO PROTECTIVE BARRIERS IN IRISH POTATO SLICES [1]

W. L. Smith, Jr.,[2] and Helen F. Smart [3]

SUMMARY

Shortly after harvest, potatoes in the United States are usually shipped or stored at temperatures near 40°F. After removal of the tubers from low temperatures, bacterial decay often develops rapidly. Slices of 6 varieties of potatoes were held in moist chambers at 40°, 50°, 60°, 70°, and 80° for 1 to 4 days. Each day tuber slices from each temperature were examined for the formation of suberin and periderm. Similar tuber slices from each temperature were inoculated daily with *Erwinia atroseptica* (v. Hall) Jennison and then placed at 70°. Results of these studies showed that at proper temperatures in moist chambers slices of the 6 varieties of potatoes formed protective barriers that were sufficient to prevent the entrance of *E. atroseptica.*

At each daily reading, development of suberin and periderm was more pronounced on slices held at 70° or 80°F than on those held at lower temperatures. Slices held for 4 days at 50° or 60° developed barriers approximately equal to those held for 2 days at 70° or 80°. Slices held at 40° developed very little suberin and no periderm during the 4-day period. Suberin and periderm developed most rapidly on slices of Irish Cobbler, followed in order by Kennebec, Katahdin, Sebago, Teton, and Mohawk.

Slices held 2 or 3 days at 70° or 80°F before inoculation developed much less decay than those held for only 1 day. Slices held at 70° or 80° for 4 days before inoculation were virtually free of decay. Decay in slices held at 50° or 60° for 4 days and then inoculated was approximately equal to decay of the slices held at 70° or 80° for only 2 days. There was no inhibition of decay on slices previously held at 40°.

The customary procedure at present is to place Irish potatoes at approximately 40°F as soon as possible after they are dug. Most growers or shippers believe that at low temperatures decay will not develop in the cuts, bruises, or skinned areas. Growers also believe that skinned areas will not dry and that the familiar surface browning and "scald" will not develop or will be reduced to a minimum. Actually there is no evidence that low temperature alone will do more than delay infection by decay-producing organisms or delay the development of surface browning. In fact, there is much evidence that rapid cooling of tubers immediately after harvest contributes to decay and surface browning (11, 13, 14, 16, 17). These disorders, though often masked while the tubers are at low temperatures, frequently develop after the tubers are removed to higher temperatures.

The purpose of the present investigation was to study the degree and rapidity of formation of protective barriers at various temperatures on wounded areas of potatoes and the possible relation of bacterial decay to the barriers.

LITERATURE REVIEW.—The basic information on the conditions necessary for healing of cut surfaces of potatoes was published many years ago, but very little practical application of the information has been made in the modern handling of potatoes. It is pertinent, therefore, that some of the earlier work be reviewed.

Among the earliest studies of protective-barrier formation of potatoes was that by Kny (4), who reported in 1889 that atmospheric moistures and oxygen are essential for rapid formation of barriers. Olufsen (5), in 1903, presented evidence that immature tubers formed protective barriers more rapidly than mature ones and that freshly harvested tubers formed barriers more rapidly and extensively than those that had been stored. He was of the opinion that there was no difference in the healing rate of injured surfaces in light and darkness. In 1906, Appel (1) reported that suberization initiated within 12 hours at 20°C formed the first barrier and that the periderm layers developed somewhat later. He was the first to point out that these layers would prevent the entrance of the blackleg organism, which he called *Bacillus phytophthorus.* Priestly and Woffenden (7) investigated the biochemical processes involved in the formation of the 2 protective barriers and reported the nature of each. They further showed that the rate of suberin development doubled with an increase in temperature from 15° to 25°C. In 1927, Artschwager (2) reported that suberin development was slowest at 2.5°C and became more rapid as the temperature was increased up to 35°. No periderm developed below 7°, and its development was most rapid at 35°. In 1938, Werner (17) studied the formation of protective barriers on different types of injured surfaces of Irish potatoes and showed the influence of temperature and humidity on rapidity of barrier formation regardless of the type of injury. In

[1] Accepted for publication July 27, 1955.

[2] Pathologist, Quality Maintenance and Improvement Section, Biological Sciences Branch, Agricultural Marketing Service, United States Department of Agriculture, Beltsville, Maryland.

[3] Formerly Associate Pathologist, Division Handling, Transportation and Storage, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland.

a recent paper, Rudd-Jones and Dowson (10) presented data which they believe showed that barriers develop more rapidly at 5° or 10° than at 25°C. They inoculated slices with *Bacterium carotovorum* before holding at various temperatures, however, and it is questionable whether they were dealing with barrier formation or the effect of the temperature on the growth of the decay-producing organism.

MATERIALS AND METHODS.—Tubers of the varieties Irish Cobbler, Kennebec, Katahdin, Sebago, Teton, and Mohawk were obtained from Maine through the courtesy of F. J. Stevenson of the U. S. Department of Agriculture, Beltsville, Maryland. All varieties were harvested on the same date and stored at Beltsville in the same room at 40°F and approximately 90 per cent relative humidity.

Rate of healing of slices from tubers of each of the varieties was determined at 3 approximately equal intervals during the 7 months the tubers were held in storage. At each examining period, tubers of all varieties were washed and dried, and then 24 slices, approximately 10 mm thick, of each variety were cut with an ethanol-flamed knife and placed in moist chambers at 40°, 50°, 60°, 70°, and 80°F. The tubers were not surface sterilized, but in no case did the noninoculated (check) slices in any of the chambers develop decay. Six slices of each variety were removed from each temperature at the end of 1, 2, 3, and 4 days.

On removal from the temperature chambers, 2 of the slices of each variety were sectioned. The other 4 slices were inoculated with a standard number of drops of 1:1000 water dilution of a 48-hour nutrient agar slant culture of *Erwinia atroseptica* (v. Hall) Jennison. This organism was used rather than the better known *E. carotovora* (L. R. Jones) Holland as it has been shown to penetrate cork barriers (12); therefore, a severe test of the inhibition of a pathogen by natural protective barriers was obtained. After inoculation, all slices were placed at 70°F. Readings were made after 2 days, and each slice was given a numerical rating based on the area decayed. The numerical decay ratings were as follows: 0, no decay; 1, small isolated spots on slice surface not penetrating sub-surface tissue; 2, decayed area spreading over approximately ⅓ of the slice surface with slight penetration into sub-surface tissue; 3, decayed area covering approximately ¾ of slice surface with medium penetration into sub-surface tissue; and 4, decayed area covering approximately entire surface of the slice and penetrating the sub-surface tissue deeply.

The presence of suberin and periderm was determined in blocks of tissue cut from the parenchyma region of the noninoculated slices of each variety from each temperature. Sections 20$\mu$ thick were cut from these blocks with a sliding microtome and placed in distilled water for 10–30 minutes. They then were stained by a modification of the stain technique described by Rose and Schomer (9). Two sections from a variety from 1 temperature were placed on a glass slide and flooded for 5 minutes with 1 per cent gentian violet in 70 per cent ethanol plus 1 or 2 drops of 28 per cent ammonium hydroxide. The sections were next rinsed with 9 per cent hydrochloric acid for 2 minutes or until clear. The slide then was flooded with 95 per cent ethanol for 10 minutes and rinsed with ethanol of the same concentration. After the ethanol rinse, the slide was rinsed twice with distilled water and kept wet until examined under a microscope. The suberized areas stained bluish or purple, and the nonsuberized cell walls remained colorless. Each slide was rated as soon as possible after staining. Periderm was rated by estimation of the thickness of the layer of cambium-like cells. Each section was rated independently by 2 workers as indicated in Table 1. The 4 ratings for each variety and temperature were averaged.

RESULTS.—The relation of barrier formation to decay at 70°F after 1-, 2-, 3-, and 4-day holding periods is illustrated in Figure 1. After 1 day, ratings for barriers formed by all the varieties were low, and the decay ratings high. As the length of the holding period increased to 4 days, barrier formation increased and decay decreased. The only important difference among varieties in barrier formation was that Irish Cobbler formed periderm more rapidly than the other varieties and that Sebago, Teton, and Mohawk were low in this respect. Varieties with high barrier ratings developed less decay than those with low barrier ratings.

Varietal variations in barrier formation ratings at different temperatures and decay ratings at 70° are shown in Figure 2. All varieties formed the greatest amount of suberin or periderm at 70° or 80°, and decay was less on slices previously held at these temperatures than on slices transferred from other holding temperatures. Slices previously held at 40° formed only a slight amount of suberin and no periderm during the 4-day holding period and had the highest decay rating. Slices held at 50° or 60° were intermediate in

TABLE 1.—*Rating systems for suberin and periderm formation*

| Rating | Suberin | Periderm |
|---|---|---|
| 0 | None | None |
| 1 | Outer cell walls stained lightly | Slight evidence of dividing cells |
| 2 | Outer cell walls showing medium-dark to dark staining | Dividing cells 1 or 2 layers deep, but very irregularly distributed. |
| 3 | Outer cell walls showing medium-dark to dark staining; stain extending 1 layer deep | Dividing cells 1 or 2 layers deep, continuous |
| 4 | Outer cell walls darkly stained; stain extending 1 or 2 layers deep | Dividing cells 2 or 3 layers deep, continuous |
| 5 | Outer cell walls stained very dark; stain extending 2 or more layers deep | Dividing cells 3 or more layers deep, continuous |

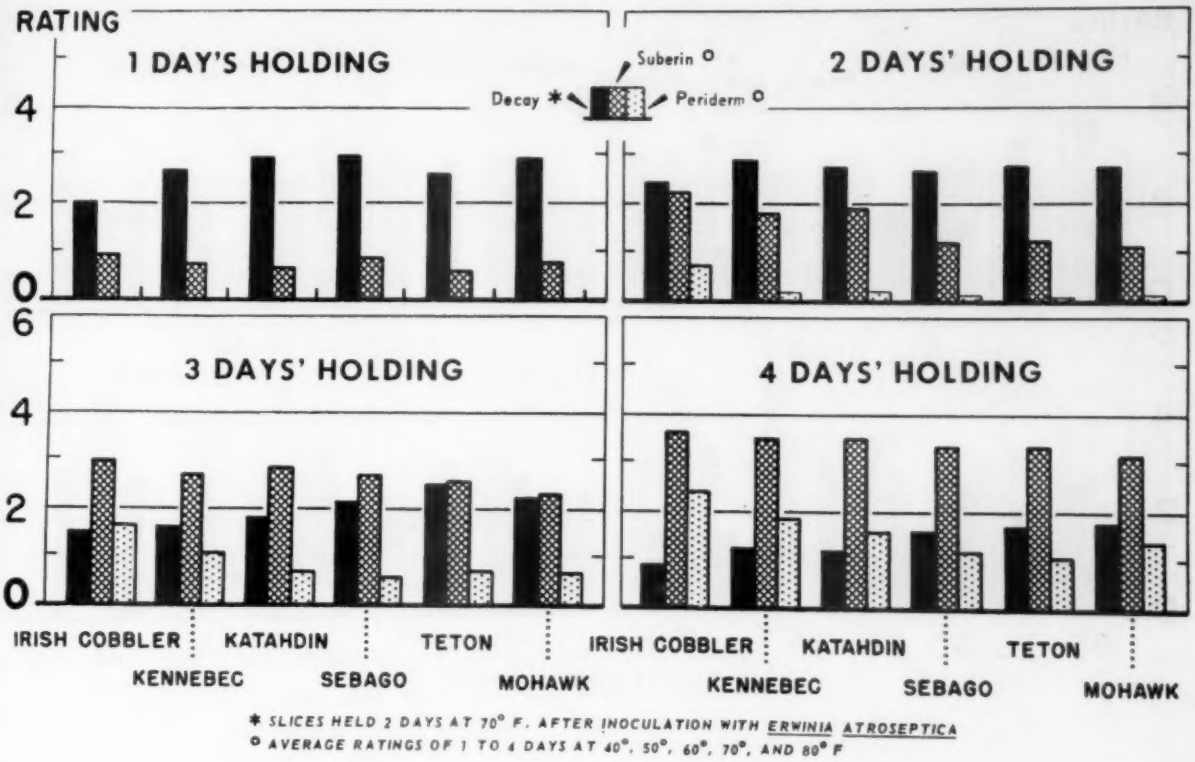



FIG. 1. Potato barrier formation and decay ratings by holding periods and variety.

both barrier formation and subsequent decay at 70°. Although varieties differed in the rapidity or extent of barrier formation, the effect of temperature on barrier development was the same for all, and the importance of the barriers in decreasing decay was consistent.

The daily ratings on healing at the various temperatures and subsequent decay of the slices at 70° are shown in Figure 3. After 1 day, the suberized layer had developed at least slightly at all temperatures except 40°, and after 2 days it was apparent at 40°. This layer gradually increased until after 4 days it was very definite at all temperatures except 40°. The development after 4 days at 40° was less than after 1 day at 70° or 80°. Development of the periderm layer did not occur at any temperature until after 2 days and then only at 70° and 80°. Periderm was evident after 3 days at 60° and very slightly evident after 4 days at 50°. It did not develop at 40° during the 4-day period.

Decay again is shown to be closely related to the barriers formed, and the barriers formed were related to the temperature and number of days. Decay was high after only 1 day regardless of the previous holding temperature, and except on slices previously held at 40°, it decreased in proportion to the amount of barrier formed at the various temperatures during the 4-day period. After 4 days' holding at 60°, the slices developed slightly greater barriers than those held at 70° or 80° for 2 days. Decay of these slices while at 70° was less than that of those held 2 days at 70° or 80°. Similarly, decay of slices held 4 days at 50° was slightly less than that of slices held 2 days at 70° or 80°. The data strongly indicate that with slightly longer exposure to any of the temperatures tested, except 40°, subsequent decay of slices when placed at higher temperatures could be virtually prevented.

The coefficients of correlation between the ratings for suberin and decay and between periderm formation and decay were extremely high (Table 2). The coefficients of determination show that 85 per cent of the variation in decay is associated with variation in the formation of suberin or periderm. The coefficient of determination between suberin and periderm indicates that 69 per cent of the variation in the 2 variables is concomitant. When suberin and periderm ratings were

TABLE 2.—*Correlation of average decay ratings of potato slices held 2 days at 70°F after inoculation with Erwinia atroseptica, and average of comparable suberin and periderm ratings after 1- to 4-day holding periods at 40°, 50°, 60°, 70°, and 80° F*

| Factors correlated | Correlation coefficients | Coefficients of determination |
|---|---|---|
| Decay rating and suberin rating | —0.92 | 0.85 |
| Decay rating and periderm rating | —0.92 | 0.85 |
| Suberin rating and periderm rating | +0.83 | 0.69 |
| Decay ratings with suberin and periderm ratings | 0.96 | 0.92 |

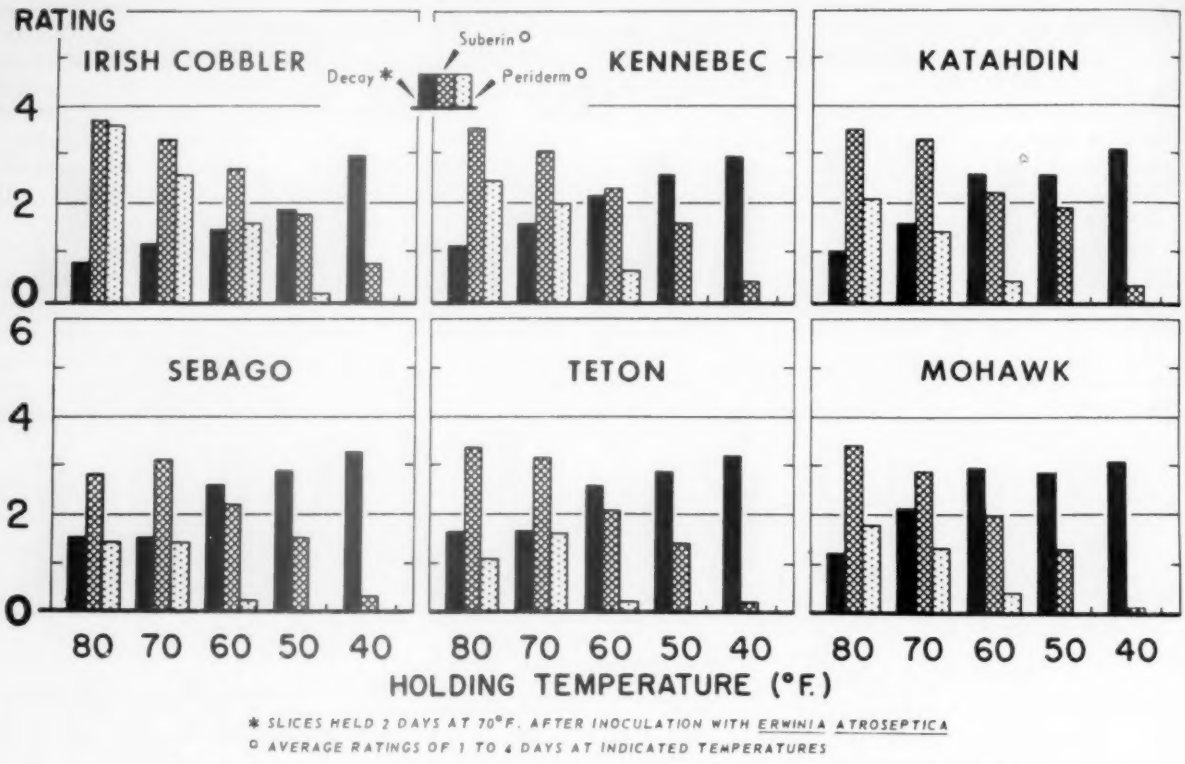



FIG. 2. Potato barrier formation and decay ratings by holding temperature and varieties.

used in a multiple correlation with decay, it was found that 92 per cent of the variation in decay is associated with variation in these barriers. Therefore, only 8 per cent of the variation of decay in these experiments may be attributed to other causes. Such a high correlation indicates that natural protective barriers are effective in reducing bacterial soft rot caused by *Erwinia atroseptica*.

DISCUSSION.—Temperatures that favor barrier formation (70–80°F) are ones at which most of the bacterial species that cause soft rot of potato tubers grow most rapidly. At such temperature, therefore, the barriers must be formed very rapidly at the injured surfaces to prevent the entrance of a pathogen. In slices that remained at 50° or 60° for 4 days, the development of the barriers and resultant decrease in decay during subsequent holding at 70° were similar to those in slices held at 70° or 80° for 2 days. At these lower temperatures, the growth of bacteria is slower than at higher temperatures. Protective barriers formed at 50° or 60° may therefore prevent the entrance of a bacterial organism at these temperatures and aid in preventing its entrance when the potatoes are later placed at a higher temperature. At 40°, the temperature commonly used in shipping or storing potatoes, there was virtually no barrier formation in 4 days. If tubers were held at this temperature, the growth of many bacterial species undoubtedly would be inhibited, but once the tubers are moved to a higher temperature the lack of any protective barrier would make them extremely susceptible to invasion by any bacterial pathogen that happened to be present.

In studies on potato propagation, Reid, Wright, and Peacock (8) demonstrated that seed pieces held at temperatures suitable for the formation of protective barriers are not invaded by seed corn maggots and have less decay than similar seed pieces held at temperatures too low for the development of protective barriers. Previously, Wright, Peacock, and Whiteman (15) had shown that freshly cut seed pieces held initially at 32° or 40°F had poorer germination than those held initially at 50°.

While working with 2 species of *Fusarium* that cause storage rots, Weiss, Lauritzen, and Brierley (16) showed that development of protective barriers in 6 varieties of potatoes differed widely, but in 1 to 4 days at 70°F the barriers formed were sufficient to inhibit the entrance of these organisms. Eight or more days were necessary before tubers held at 50° were able to escape invasion by these organisms.

A number of studies have been conducted on nonpathological disorders or general appearance of the potato as affected by protective barriers. Wright, Peacock, and Whiteman (14) reported in 1927 that if freshly harvested tubers were stored immediately at 32° or 40°F, shriveling and darkening of the wounded areas occurred. Tubers from the same source stored initially at 50°, 60°, or 70° showed considerably less of this type of injury and appeared nearly normal. In a later report (6), they indicated that the lack of pro-

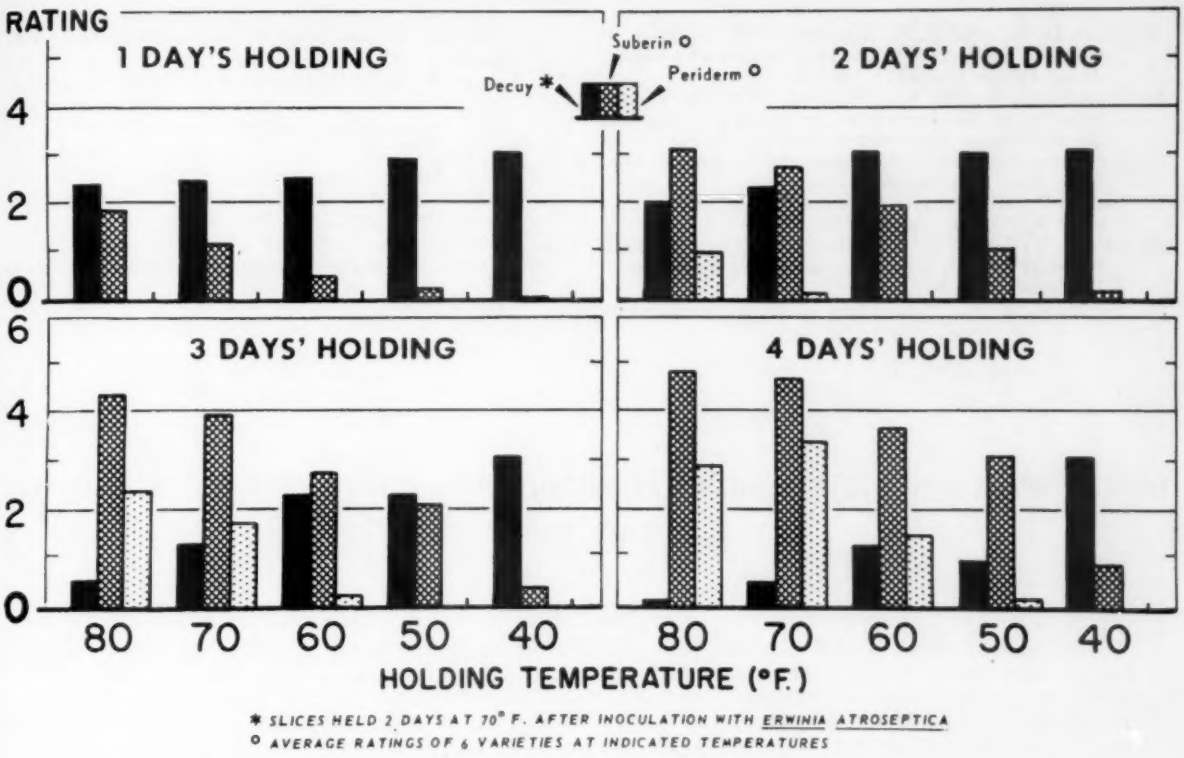



FIG. 3. Potato barrier formation and decay ratings by holding periods and holding temperatures.

tective barriers may be one of the reasons why the injured surfaces blacken when they are exposed to the sun or drying winds. More recently, W. Smith (13) showed that tubers held at 40° and then moved to a higher temperature had more surface browning on skinned areas than tubers held at 60° or 70° before they were changed to the same higher temperature. Similar results were obtained by Barger et al (3) in a transcontinental shipping test of new potatoes. They showed that tubers that had been placed in cars precooled so that the tuber temperatures were lowered rapidly to 40° developed more surface browning at room temperature than similar tubers allowed to cool slowly to approximately 50°. Similar use of the information on protective barriers was made by O. Smith (11) in studies on storage of potatoes in 1933. His data showed that tubers conditioned at 65°–68°F before storage at 45°–46° lost less weight and developed less decay than those that were at 45°–46° constantly.

The data presented here show that protective barriers, sufficient to prevent the entrance of a bacterial species that causes decay, will form on slices of potatoes within 4 days at temperatures as low as 50°F. They agree with previously published data of some other workers. It would seem desirable, therefore, to hold potatoes at temperatures suitable for protective-barrier formations for a short period prior to placing them in cold storage. A period of about 4 days at 55°–60°F is suggested.

U. S. PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. APPEL, O. 1906. Zur Kenntniss des Wund-Verschlusses bei den Kartoffeln. Ber. Deutsch. Bot. Ges. **24**: 118-122.
2. ARTSCHWAGER, E. 1927. Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agr. Res. **35**: 995-1000.
3. BARGER, W. R., J. M. HARVEY, W. A. RADSPINNER AND L. L. MORRIS. 1952. Transportation tests with early potatoes from Kern County, California to New York City, May 1951. U. S. Dept. Agr., Bur. Pl. Indus., Soils and Agr. Engin. Div. Handling, Transp. and Storage Off. Rpt. 271, 23 p.
4. KNY, L. 1889. Über die Bildung des Wundperiderms an Knollen in ihrer Abhangigkeit von ausseren Einflussen. Ber. Deutsch. Bot. Ges. **7**: 154-168.
5. OLUFSEN, L. 1903. Untersuchungen über Wundperidermbildung an Kartoffel Knollen. Bot. Centralbl. Reich. **15**: 269-308.
6. PEACOCK, W. M., R. C. WRIGHT, AND T. M. WHITEMAN. 1931. The Relation of solar and sky radiation, temperature, and humidity to the sun scald of potatoes in 1931. Amer. Soc. Hort. Sci. Proc. **28**: 261-265.
7. PRIESTLEY, J. H., AND LETTICE M. WOFFENDEN. 1923. The healing of wounds in potato tubers and their propagation by cut sets. Ann. Appl. Biol. **10**: 96-115.
8. REID, W. J., JR., R. C. WRIGHT, AND W. M. PEACOCK. 1940. Prevention of damage by the seed corn maggot to potato seed pieces. U. S. Dept. Agr. Tech. Bul.

719, 37 p.
9. Rose, D. H., and H. A. Schomer. 1944. Relation of heat and dessication to bacterial soft rot of potatoes. Amer. Potato Jour. **21**: 149-161.
10. Rudd-Jones, D., and W. J. Dowson. 1950. On the bacteria responsible for soft rot in stored potatoes, and the reaction of the tuber to invasion by Bacterium carotovorum (Jones) Lehmann and Neumann. Ann. Appl. Biol. **37**: 563-569.
11. Smith, O. 1933. Studies of potato storage. N. Y. (Cornell) Agr. Expt. Sta. Bul. 553, 57 p.
12. Smith, W. L., Jr. 1950. Pathogenic differences manifested by Erwinia atroseptica and Erwinia carotovora. Phytopathology **40**: 1011-1017.
13. Smith, W. L., Jr. 1952. Effect of storage temperature, injury and exposure on weight loss and surface discoloration of new potatoes. Amer. Potato Jour. **29**: 55-61.
14. Wright, R. C., W. M. Peacock, and T. M. Whiteman. 1927. The storage of potatoes immediately after harvest. Potato Assoc. Amer. Proc. **14**: 122-125.
15. Wright, R. C., W. M. Peacock, and T. M. Whiteman. 1934. Effect of subsequent yields on storing cut seed potatoes at different temperatures and humidities. U. S. Dept. Agr. Tech. Bul. 394, 20 p.
16. Weiss, F., J. I. Lauritzen, and P. Brierley. 1928. Factors in the inception and development of Fusarium rot in stored potatoes. U. S. Dept. Agr. Tech. Bul. 62, 36 p.

# HOST-PENETRATION AND PATHOLOGICAL HISTOLOGY IN GRAY LEAF SPOT OF TOMATO[1]

Urban L. Diener[2]

SUMMARY

Histological studies showed that the pathogen, *Stemphylium solani*, invaded the tomato leaf primarily through the stomates, although direct penetration also was found. The fungus entered the stomate by an infection peg or by the germ-tube tip. Direct penetration occurred by infection pegs entering between epidermal cells or between the outer guard-cell wall and the adjacent epidermal cell.

The development of the fungus in the leaf tissues was the same, regardless of the mode of penetration or the leaf surface invaded. Bulbous primary hyphae developed from the infection hyphae in the substomatal or other subepidermal cavities within 24 hours after inoculation. Secondary hyphae originated from the primary hyphae and ramified intercellularly through the host tissue. Extensive development of secondary hyphae was found 48 hours after inoculation.

About 60 hours after inoculation, host cell protoplasts in the lesion appeared disorganized, and a dense granular material was evident. Disintegration of the cells occurred in the immediate area of the invading hyphae. Cell walls in advance of the hyphae were visibly altered. Peripheral growth of the fungus appeared to cease in the later stages of infection.

Gray leaf spot of tomato (*Lycopersicon esculentum* Mill.), incited by *Stemphylium solani* G. F. Weber, has become widespread and destructive during the past 15 years in certain areas of the United States. Attention became focused on this disease in North Carolina in 1948 and 1949, when it caused severe defoliation of tomato plants in experimental plantings of breeding lines. An investigation of the disease, with the emphasis on host-parasite relationships, was initiated after a method was developed for producing abundant conidia in pure cultures of the causal organism (3, 4). Host penetration and the pathological histology of the disease in tomato leaf tissues were studied.

Materials and methods.—Louisiana Pink and Marglobe, susceptible tomato varieties, were selected for the study of host-parasite relationships. The culture of *Stemphylium solani* designated as O-5 (4) was used.

Inoculum was prepared from sporulating cultures. Only the sporulating zone was used. The conidia were floated off in distilled water by gently rubbing with a camel's-hair brush or glass rod. The spore suspension was filtered through cotton batting to remove mycelial fragments and then concentrated by centrifuging. The inoculum was later diluted with water to a uniform concentration.

Inoculations were completed 2 hours after the inoculum was prepared. A drop of inoculum containing 12–15 conidia per low-power-microscope field (× 100) was placed on either the upper or lower leaf surface in areas delimited with India ink. Inoculated plants were

[1] Accepted for publication July 29, 1955.
A portion of a thesis submitted to the graduate faculty of North Carolina State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology. The writer is indebted to his advisor, Dr. D. E. Ellis, and to Drs. C. J. Nusbaum and L. W. Nielsen for their assistance during the period of the work and for criticisms of the manuscript.
Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 652 of the Journal Series.
[2] Assistant Plant Pathologist, Alabama Polytechnic Institute, Auburn, Alabama. Formerly Graduate Assistant in Plant Pathology, North Carolina Agricultural Experiment Station.

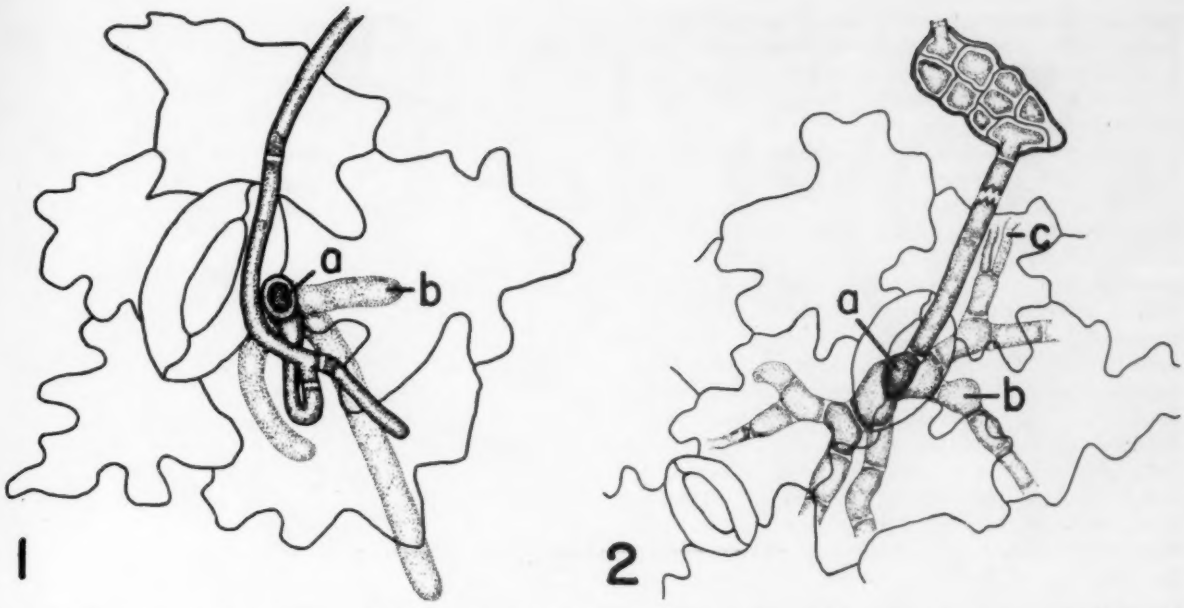



FIG. 1-2. Penetration and infection of tomato leaves by *S. solani*.—Fig. 1. Direct penetration from cleared-leaf mounts (×800). a) Infection hypha, b) primary hypha.—Fig. 2. Stomatal penetration from cleared leaf mounts (×600). a) Infection hypha, b) primary hypha, c) secondary hypha.

placed in a humidity chamber, and after definite intervals the inoculated areas were cut from the leaves with a no. 6 cork borer and the discs of leaf tissue placed in a fixative.

Discs of inoculated tissue, for studies of leaf penetration *in situ*, were removed 3, 6, 9, 12, 15, 18, 21, 24, and 40 hours after inoculation and killed and fixed in acetic-alcohol (equal parts of 95 per cent ethyl alcohol and glacial acetic acid). Discs were cleared by a modification of the method described by Peace (8). After 2 days in acetic-alcohol, the discs were placed in 70–75 per cent lactic acid (2). After 4–8 days at room temperature (27°C) or at least 2 days at 40°C, the discs were removed from the clearing agent. A drop or 2 of 0.1 per cent cotton blue in lactophenol was placed on a disc on a slide after removal of the free lactic acid with blotting paper. After 5–30 minutes, the excess cotton blue was removed by flooding with clear lactophenol. The fungus stained blue while the host tissues remained clear. Restaining a few hours later or the next day intensified the stain in the fungus and improved differentiation. Cleared but unstained discs and stained discs were bottled in clear lactophenol for later observations.

Discs of inoculated tissue for sectioning were collected 12, 24, 36, 48, 60, and 72 hours after inoculation and killed and fixed in FAA (95 per cent ethyl alcohol, 100 ml; glacial acetic acid, 5 ml; 37 per cent formalin, 13 ml). Dehydration was by the n-butyl alcohol method (11). Leaf tissue infiltrated first with paraffin and then with "Tissuemat"[3] was cut in serial cross-section 9–10μ thick, affixed to slides with Adhesive III (10), and stained with safranin and fast green (6).

RESULTS.—In stomatal penetration, the germ tubes entered the stomates by means of the development of an infection hypha or peg near the tip of the germ tube or the tip of a side branch from a germ tube. Evidence of penetration was most readily observed in cleared leaves stained with cotton blue. The infection peg or hypha usually stained a more intense blue than the germ tube. In numerous instances, a slight swelling of the germ-tube tip was observed that possibly preceded the development of the infection peg. In other cases, however, a swelling of the germ tube was not observed, and the tube apparently entered the stomate without forming a dense-staining infection peg.

Occasionally, evidence of direct penetration of the lower leaf surface of Marglobe tomato was observed in material fixed 40 hours after inoculation. A swelling of germ-tube tips appressed to the leaf surface previously had been noted in other material. With these preparations, however, infection peg and subepidermal development occurred. Germ-tube tips appressed to the leaf surface swelled to give an appressorium-like structure. The infection peg developed on the under side of the swelling. In none of the material studied had this peg pierced the epidermal cell, but it appeared to have entered at the juncture of 2 epidermal cells or between the outer guard-cell wall and the adjacent epidermal-cell wall (Fig. 1). In this case the peg had entered at the juncture of the epidermal-cell walls and the outer guard cell.

After the infection peg had reached a substomatal cavity or other subepidermal opening, the develop-

[3] A special embedding paraffin (MP 54–56°C) compounded by Fisher Scientific Company.

ment of the fungus proceeded in a similar manner following either stomatal or direct penetration. Since stomatal entrance appeared to be the dominant mode of penetration, development of the fungus following stomatal entry is described.

The infection peg or hypha usually expanded to form bulbous enlargements or vesicles (the primary hypha) in the substomatal cavity or adjacent opening. Several of these thick cells formed successively into 1 or several dichotomous branches. Septations were quite distinct. The protoplasm within the primary hyphal cells stained heavily. Primary hyphae were commonly noted in material fixed 12–24 hours after inoculation. Slender, rapidly growing intercellular hyphae originated from these stubby primary hyphal branches. The intercellular or secondary hyphae ramified throughout the spongy parenchyma cells. Details of stomatal penetration, primary hyphal formation, and the initiation of the secondary hyphae, as observed from cleared-leaf mounts, are illustrated in Figure 2. In some cases the fungus did not form an infection peg or bulbous primary hypha in the substomatal area, but it quickly ramified and developed intercellularly among the spongy parenchyma cells in the manner of secondary hyphae.

Certain details of penetration and the initiation of infection, as noted in cleared leaves, also were observed in stained serial sections. Stomatal penetration by an infection peg and subsequent primary and secondary hyphal development are illustrated in Figure 3, a composite camera lucida drawing from several slides. The infection peg stained more heavily with safranin than did adjoining parts of the fungus. This was the greatest extent of the development of the fungus observed in tissue fixed within 24 hours after inoculation. Figure 3 and the description of the development of the fungus in the host tissues were made from inoculated upper surfaces of leaves. Penetration and development of the fungus within the host tissues from inoculated lower surfaces appeared to have proceeded in a similar manner.

Host-parasite reactions were most clearly defined in serial cross sections of material fixed 48, 60, and 72 hours after inoculation. Cleared-leaf mounts fixed at 40 hours showed slight changes in staining properties in the areas adjacent to the penetrating fungus, but well-defined morphological changes were not evident. Evidence of host-parasite reactions were observed in serial sections of Louisiana Pink leaf tissue inoculated

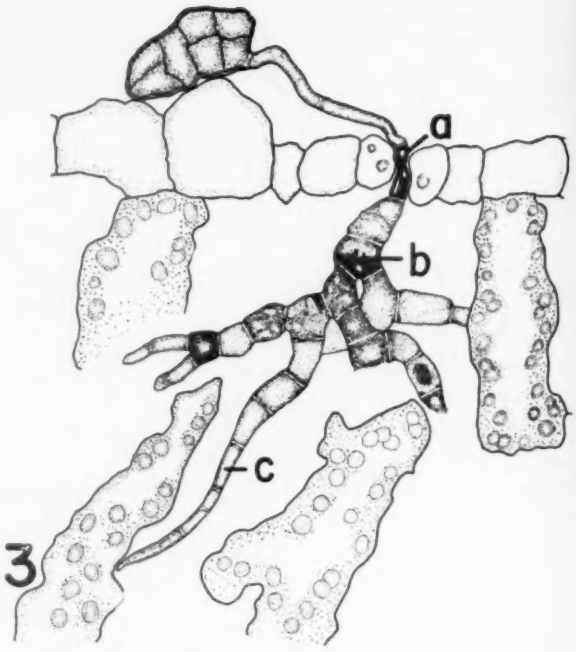
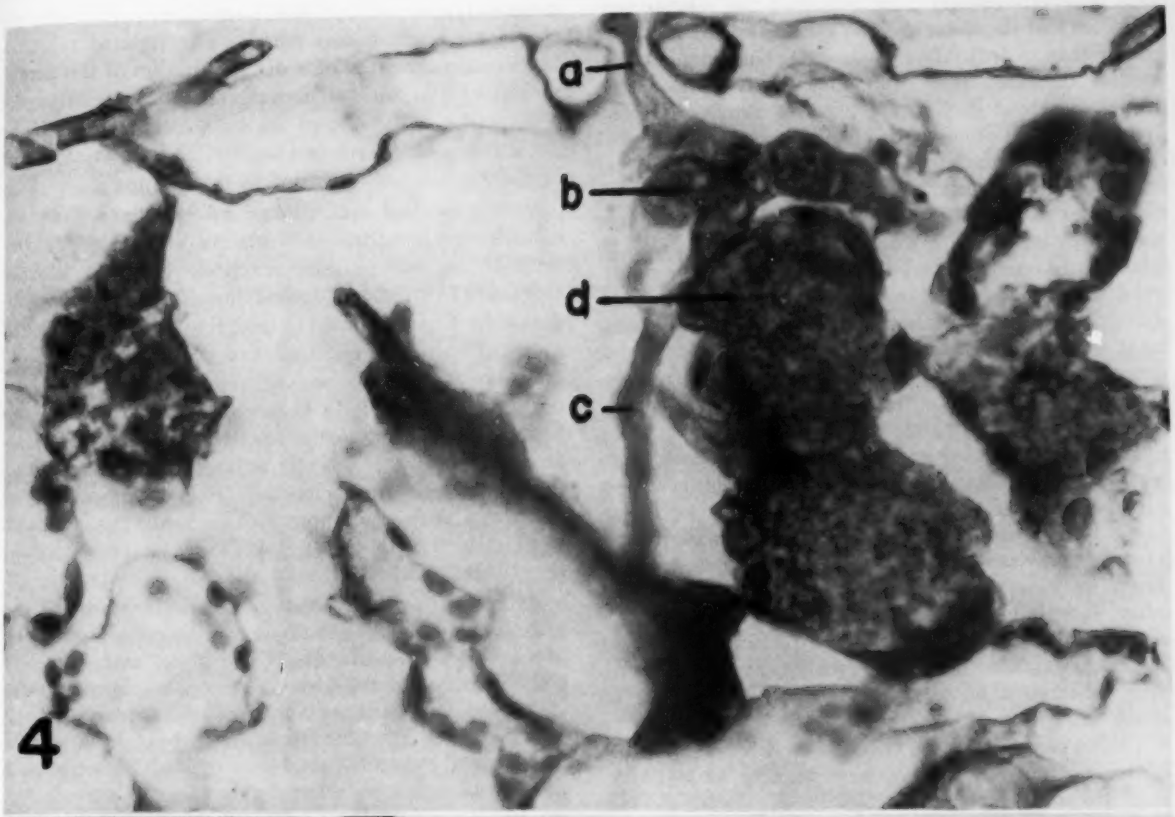


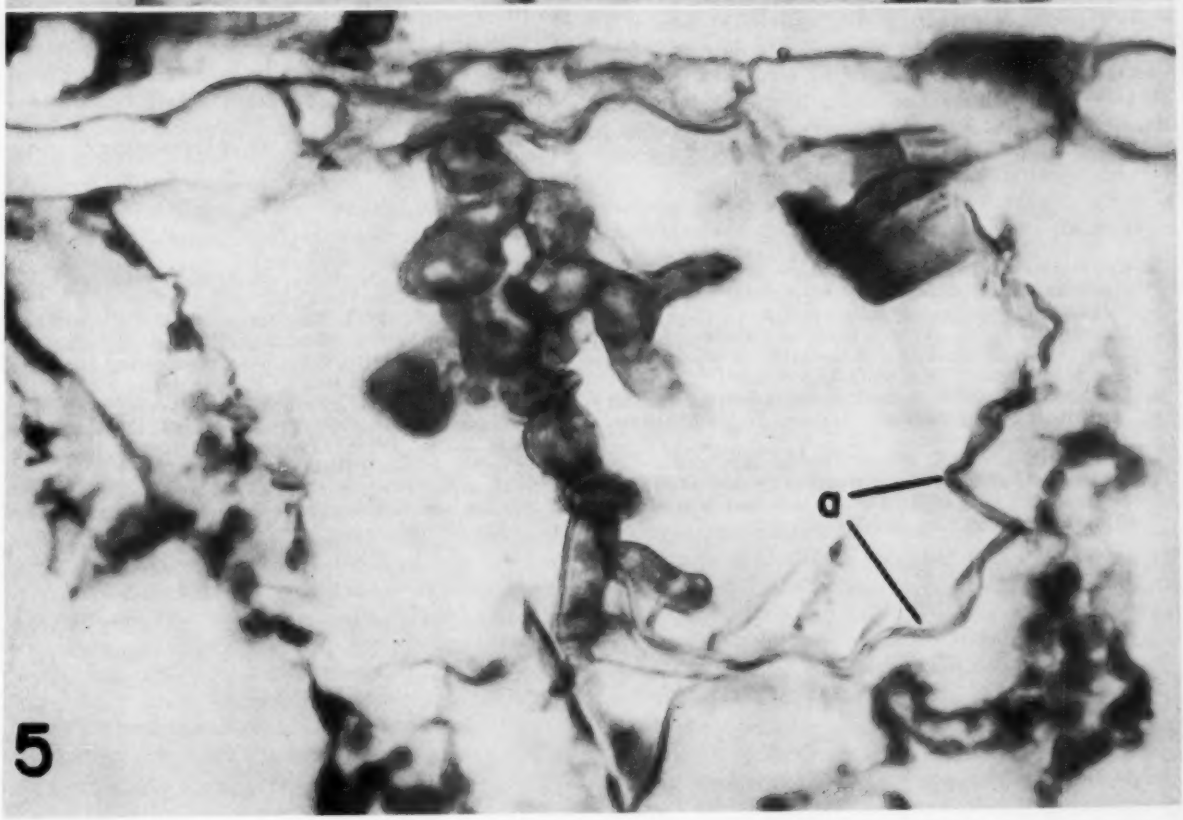

FIG. 3. Stomatal penetration from serial sections (×500). a) Infection hypha, b) primary hypha, c) secondary hypha.

on the upper leaf-surface and fixed at 60 hours after inoculation (Fig. 4). The leaf tissue obviously was invaded through the stomate since the infection peg in the stomatal opening is well-defined. Primary and secondary hyphal development of the fungus also is visible. The chloroplasts in the palisade cell near the invaded stomate are disintegrated and disorganized, and the cytoplasm has a granular appearance. The cell walls and protoplasm are stained heavily with safranin. Adjacent palisade cells also appear to have been affected as indicated by the change in staining properties of the host cells to safranin. Knotty fungus hyphae developed in the center of the palisade layer (Fig. 5). Few host cells are evident in the immediate vicinity of the fungus. The cell walls above and around the fungus (a) are heavily stained and appear different in thickness and organization from the walls of normal host cells.

DISCUSSION.—Under field conditions, the Louisiana Pink tomato variety was rated more susceptible to *Stemphylium solani* than the Marglobe variety. These

FIG. 4-5. Host-parasite reactions in tomato leaves from serial sections, 60 hours after upper leaf-surface inoculation.—Fig. 4. Advanced stage of infection; a) infection peg in stomate; b) primary hypha; c) secondary hypha; d) host cell reaction to the fungus, disorganized chloroplasts, granular cytoplasm.—Fig. 5. Fungus hypha constricted. a) Cell walls collapsed and altered as a result of the host-parasite interaction.


a
b
d
c
4


a
5

field observations may have evaluated the relative susceptibility of the 2 varieties to defoliation by *S. solani* rather than to leaf infection, as greenhouse inoculation studies indicated little difference between the 2 varieties. In these studies, the greatest number of infections developed from lower leaf-surface inoculations.

In 1908, Eckerson (5) reported that the number of stomates on the lower leaf-surface exceeded those on the upper surface by about 10 to 1. Extensive counts and observations were made of the stomates on Marglobe and Louisiana Pink tomato leaves, and the difference was about 3 to 1 in favor of the lower leaf-surface. Therefore, with stomatal entrance the predominant mode of penetration by the gray leaf spot fungus, greater infection might be expected to occur from inoculations on the lower leaf-surface than from inoculation on the upper surface.

In a few cases, vegetative growth developed from mycelial fragments, but no evidence was observed on cleared leaves of penetration from this type of inoculum. The inoculum was almost entirely conidial, however, and evidence of stomatal penetration was readily found in cleared-leaf preparations fixed 6–12 hours after inoculation. Direct penetration is possibly a slower process than stomatal penetration, as in only 1 instance was subepidermal development following direct penetration noted in material fixed less than 40 hours after inoculation.

Cunningham (1) made a comprehensive study of the host-parasite relations of a large number of leaf spotting diseases which he placed into 2 groups. Certain hosts (species of *Prunus*, *Pyrus*, and *Beta*) formed a cicatrice when attacked by necrogenic pathogens. Meristematic activity was initiated in mesophyll cells in advance of the fungus, resulting in the occlusion of intercellular spaces and the formation of a wound periderm with a layer of cork cells walling off the fungus. In the second and larger group of hosts, no cicatrice formation was noted. The type of reaction in this case consisted of a disorganization of the protoplasm, with or without dense granular material in the cells, and a collapsing of the cells as noted for *Alternaria solani* on potato and tomato. No suberization of host cell walls was recorded in this group.

With gray leaf spot disease on Louisiana Pink tomato leaves, no cicatrice formation was observed. Details of the host-parasite reactions (Fig. 4) closely correspond to those described for *Alternaria solani* on potato by Cunningham (1) and Rands (9). The palisade cells are disorganized and cell contents have a granular appearance, and adjoining cells appear partially disintegrated. In Figure 5, disintegration of the host cells adjacent to the knotted, constricted fungus is apparent. Walls of cells adjacent to this area also appear to have been changed. Beyond these altered cell walls, the tissue appears to be healthy. Leaf sections stained with Sudan III showed no evidence of suberin in the affected cell walls.

After the initial penetration, the development of the gray leaf spot fungus is rapid in a seemingly compatible host for 36–48 hours. Between 48 and 60 hours after inoculation, the first visible symptoms of disease indicating cell collapse and necrosis appear. Leach (7) describes a similar situation in bean anthracnose in which a congenial symbiosis existed between host and parasite, but with the development of the secondary hyphae a lytic effect followed, resulting in host cell collapse and in the appearance of macroscopic necrotic lesions.

PLANT PATHOLOGY
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
RALEIGH, NORTH CAROLINA

LITERATURE CITED

1. CUNNINGHAM, H. S. 1928. A study of the histologic changes induced in leaves by certain leaf-spotting fungi. Phytopathology **18**: 717-751.
2. DEBENHAM, E. M. 1939. A modified technique for the microscopic examination of the xylem of whole plants or plant organs. Ann. Bot. **3**: 369-373.
3. DIENER, U. L. 1952. A method for inducing abundant sporulation of Stemphylium solani in pure culture. Phytopathology (Abs.) **42**: 7.
4. DIENER, U. L. 1955. Sporulation in pure culture by Stemphylium solani. Phytopathology **45**: 141-145.
5. ECKERSON, S. F. 1908. The number and size of stomata. Bot. Gaz. **46**: 221-224.
6. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw Hill, New York. 523 p.
7. LEACH, J. G. 1923. The parasitism of Colletotrichum lindemuthianum. Minn. Agr. Exp. Sta. Bul. 14, 57 p.
8. PEACE, L. M. 1910. Notes upon the clearing and staining of leaves and stems. Pl. World **13**: 93-96.
9. RANDS, R. D. 1917. Early blight of potato and related plants. Wis. Agr. Exp. Sta. Res. Bul. 42, 48 p.
10. SASS, J. E. 1940. Elements of botanical microtechnique. McGraw Hill, New York. 222 p.
11. ZIRKLE, C. 1930. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science **71**: 103-104.

# A STUDY ON THE MODE OF ACTION OF THE WILDFIRE TOXIN [1]

Armin C. Braun

SUMMARY

The results of physiological and chemical studies are presented which indicate that the bacterial toxin associated with the wildfire disease of tobacco is a structural analogue of methionine that owes its biological activity in one and perhaps all susceptible plant species to its behavior as a naturally occurring antimetabolite of that essential amino acid.

The present study was designed to define in physiological and biochemical terms the course of events leading to the production of a specific pathological lesion. In such an analysis, it is essential to learn not only the chemical structure of all components participating in the causation of the lesion but also to attempt to determine from the basis of that structure the mechanism involved in the development of the resulting pathology. Only if this is achieved can an intellectually satisfying account of a disease process be arrived at. The present paper is a review of work that has been done on a relatively simple system, in which a number of components concerned in the causation of 1 phase of a naturally occurring plant disease are defined in physiological and biochemical terms.

In 1917 a bacterial leafspot disease of tobacco was described by Wolf and Foster (17) as occurring in North Carolina. This disease is now found in most tobacco-growing regions of the world. Because of the suddenness of its appearance, the rapidity of its spread, and the general severity of the disease in seedbed and field, the name "wildfire" was applied to it by tobacco growers. The symptoms of the malady are conspicuous and, once observed, cannot easily be confused with any other leafspot disease of tobacco. Wildfire is characterized by localized chlorotic halos 1–2 cm in diameter that surround a central brown necrotic spot. These yellow halos are free of bacteria and result from the diffusion of a toxic substance secreted by bacteria that are present in the central necrotic foci of infection. In small seedbed-grown plants, the toxin may become systemic, causing stunting and ultimate death of affected plants.

The wildfire bacterium, *Pseudomonas tabaci* (Wolf & Foster) Stevens, produces this toxin on a wide variety of culture media as well as in the host. Johnson and Murwin (10) first demonstrated that sterile Berkefeld filtrates of a bacterial culture can reproduce the toxic manifestations of the wildfire disease not only in tobacco but also in a large number of plant species representing many different families. This finding has been fully confirmed (4). When the wildfire bacterium loses its ability to secrete the toxin, it becomes indistinguishable from the angular leafspot organism of tobacco, *Pseudomonas angulata* (Fromme & Murray) Holland, in disease-producing ability (2).

The wildfire toxin is admirably suited for physiological and biochemical studies on the causation of disease. The lesions it produces are so characteristic that there can be little doubt that the toxin produced by the bacteria in the host tissue is the same substance as that found in the culture filtrate. The toxin is elaborated by the bacteria in short periods of time in a simple inorganic salts-sucrose medium. It can be assayed in a roughly quantitative manner, and under suitable conditions the results of the assay are fully developed in less than 48 hours. The wildfire toxin is not selective, and the cells of many plant species are susceptible to its action.

In studying the mode of action of the wildfire toxin it was found advantageous to transfer the initial experiments from the level of the whole plant to that of free-living plant cells. The alga *Chlorella vulgaris* (Beijer.), which was used in these investigations, offered several advantages over the whole plant as a test object. This organism was used because 1) it is susceptible to the action of the toxin; 2) the cells of the alga multiply profusely under suitable cultural conditions, whereas the cells of a plant leaf are essentially resting cells; 3) it can be grown readily on a simple chemically-defined culture medium, and growth rates can be measured quantitatively; and 4) the alga is bathed by the nutrient solution in which it develops, and therefore concentrations of the metabolites and the toxin can be accurately controlled.

The chlorotic halo that develops in the plant leaf as a result of the action of the wildfire toxin is clearly concerned either with the destruction of chlorophyll by the toxin or with the inability of the plant to synthesize normal amounts of chlorophyll in the presence of the toxin. Spectroscopic analysis and chemical studies of chlorophyll present in toxin-treated leaf material and in comparable normal tissues showed that the concentration of chlorophyll in the chlorotic tissue is considerably less than that present in normal leaf tissue of the same type and that the chlorophyll molecule in both instances is intact. Derivatives of chlorophyll, such as phaeophytin, were not detectable spectroscopically as might possibly be expected if the toxin exerted its biological effects directly on the chlorophyll molecule.

When the wildfire toxin was tested against Chlorella, the growth of that organism was completely inhibited in the presence of suitable concentrations of sterile toxin (3). Furthermore, when the toxin-containing medium was supplemented with liver or yeast extract,

[1] Accepted for publication July 26, 1955.

a complete reversal of the deleterious effects of the toxin on the growth of Chlorella was obtained. This finding suggested that the toxin interfered with the synthesis by Chlorella of some essential growth factor or that it impaired the ability of the organism to utilize such a factor. When a large number of compounds known to be present in the liver and yeast extracts were tested individually, only dl-methionine was capable of completely negating the action of the toxin when relatively high concentrations of the latter were present in the culture medium. Of the optical antipodes of methionine, only the l or natural form was found to reverse the toxic action. Methionine did not inactivate the toxin.

In recent years, considerable progress has been made in characterizing the metabolic pathway for the biosynthesis of certain amino acids in microorganisms. One probable pathway of methionine synthesis, i.e. cysteine → cystathionine → homocysteine → methionine, has been established for several species of organisms including *Bacillus subtilis* Cohn emend. Prazmowski (16), *Escherichia coli* (Migula) Castell. & Chalmers (11, 15), and *Neurospora crassa* Shear & Dodge (9). When tested separately in the presence of the toxin, however, the 3 intermediates were found to be incapable of overcoming the deleterious effects of the toxin on the growth of Chlorella. If, as seems likely, the synthesis of methionine in Chlorella follows a pattern similar to that described for the other organisms studied, then the toxin would appear to exert its biological effects either by blocking the methylation of homocysteine, the immediate precursor of methionine, or by interfering with the utilization of the methionine that is normally synthesized by Chlorella. In testing the first possibility, it was found that when homocysteine was used in conjunction with either choline or betaine, 2 excellent methylating agents, in the presence or absence of para amino benzoic acid, a reversal of the growth-inhibiting effect of the wildfire toxin on Chlorella was not accomplished. This suggests, but it is by no means conclusive evidence, that the toxin does not interfere with the methylation of homocysteine. Transmethylations may be complex reactions. In Neurospora, for example, it has been found (5) that 2 and possibly as many as 4 different genetically controlled reactions may be involved in this 1 step alone.

There is, however, another method available for determining whether the effect of the toxin is on homocysteine, the immediate precursor of methionine, or whether the toxin competes with methionine for active centers on the enzyme that normally combines with and acts on methionine.

Growth inhibitors such as the wildfire toxin have commonly been considered to interfere either with the synthesis or with the utilization of an essential metabolite. If, for example, the synthesis of methionine were affected by virtue of the fact that homocysteine is not methylated in the presence of the toxin, then the minimum concentration of methionine that reverses the action of a given concentration of toxin should also suffice to reverse any reasonable increase in the concentration of the toxin. If, on the other hand, the utilization of methionine by Chlorella is affected by the action of the toxin, a more or less constant ratio would persist between the toxin and methionine over a wide range of concentrations. When data obtained in experiments in which 3 different concentrations of chemically pure toxin tested against a range of concentrations of l-methionine were plotted, it was found that the resulting curves were characterized over a range of concentrations by a more or less constant ratio between toxin and methionine (Fig. 1). These results suggest, therefore, that the toxin competes with methionine for active enzyme groupings thereby preventing normal utilization of this essential amino acid by Chlorella. A unique type of nutritional deficiency is thus created.

Although the competitive reversal of the toxic action by methionine was clearly evident in the case of Chlorella, a similar reversal was not accomplished when tobacco leaves were treated with methionine either by immersing the affected leaves in a solution of that amino acid or when methionine was infiltrated into a leaf before, simultaneously with, or after the toxin was introduced into the leaf. Other substances

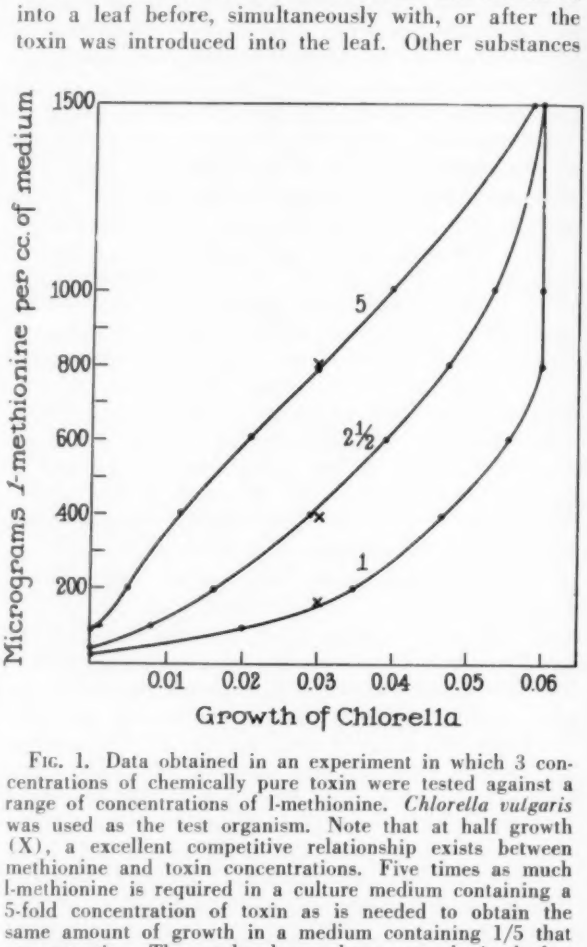



FIG. 1. Data obtained in an experiment in which 3 concentrations of chemically pure toxin were tested against a range of concentrations of l-methionine. *Chlorella vulgaris* was used as the test organism. Note that at half growth (X), a excellent competitive relationship exists between methionine and toxin concentrations. Five times as much l-methionine is required in a culture medium containing a 5-fold concentration of toxin as is needed to obtain the same amount of growth in a medium containing 1/5 that concentration. The results shown above were obtained after a 3-day incubation period at 25°C.

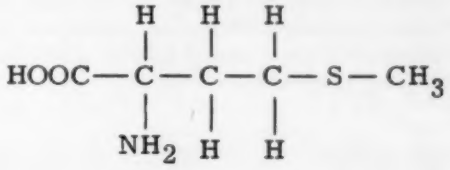
or combination of substances such as those found in liver and yeast extracts or in enzymatic and acid hydrolyzed casein were also ineffective in reversing the chlorotic effects of the toxin in a tobacco leaf. These results suggested either that the mechanism of action of the wildfire toxin differed in the 2 forms of plant life or that the action was the same but that a reversal of the toxic effect in the plant leaf with dl- or l-methionine was not possible under the conditions of the experiment.

The experimental results obtained with the use of Chlorella nevertheless indicated that the wildfire toxin is a structural analogue of methionine and owes its biological activity in plants generally to its behavior as a naturally occurring antimetabolite. This hypothesis could best be tested by isolating the wildfire toxin in pure form and determining its structure. It also could be tested, in perhaps a somewhat less satisfactory manner, by ascertaining whether any of the known structural analogues of methionine would, when introduced into tobacco leaves, reproduce the toxic manifestations of the wildfire disease. Both approaches were used in this study.

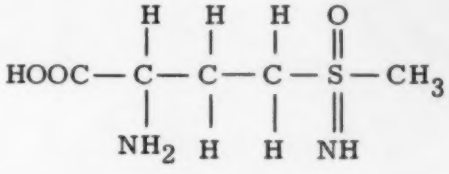
At about the time that the present investigation was getting under way, it was reported from England that dogs and certain other animals fed on a ration containing a high proportion of milled but not of unmilled wheat flour developed convulsive seizures known as running fits. In order to improve the baking quality of flour, the wheat is commonly agenized during the milling process by treating it with nitrogen trichloride. The nitrogen trichloride was shown to react with wheat proteins to form the toxic agent responsible for the canine hysteria. Reiner et al (14) postulated on the basis of analytical data that the toxic factor was a derivative of methionine. These investigators then demonstrated (14) that the convulsant action of the toxic factor could be overcome in rabbits and mice with methionine. The toxic agent was found almost simultaneously by 2 groups of workers (1, 13) to have an empirical formula of $C_5H_{12}N_2O_3S$, the structure of which is shown in Figure 2. This compound, methionine sulfoximine, also has been shown to inhibit the growth of *Leuconostoc mesenteroides* (7), and this inhibition has been reversed by both methionine (12) and glutamine (8).

When methionine sulfoximine was introduced in aqueous solution in concentrations of from 0.05 to 0.1 mg/ml of water into tobacco leaves, it was found to elicit the production of chlorotic halos that were very similar to if not indistinguishable from those produced by the wildfire toxin (Fig. 3). Ethionine sulfoximine was found to behave similarly. Other structural analogues of methionine tested, however, were ineffective in eliciting the formation of chlorotic halos in tobacco (Fig. 3). Thus, structural analogy does not necessarily make a compound an antimetabolite. The antimetabolite must be able to exclude, because of greater affinity, its structurally related metabolite from the specific cellular reaction that leads, as a result of a deficiency of the metabolite, to the development of the pathological lesion.

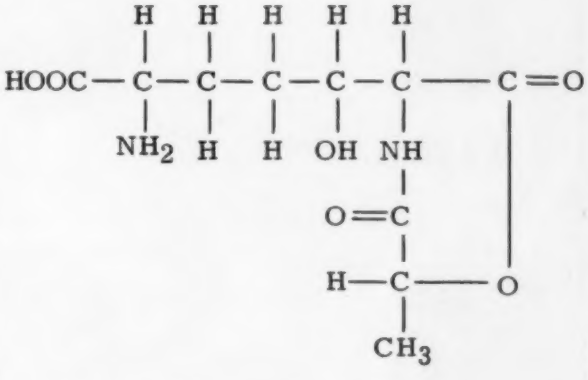
Since methionine sulfoximine is a known structural analogue of methionine that is capable of reproducing the toxic manifestations of the wildfire disease, a comparative study of the biological activity of this substance and the wildfire toxin was undertaken. Like the wildfire toxin, methionine sulfoximine inhibited the growth of Chlorella in culture, and this inhibition was reversed competitively with l-methionine. l-Glutamine did not reverse the toxic effects of methionine



METHIONINE



METHIONINE SULFOXIMINE



WILDFIRE TOXIN

FIG. 2. The structures of methionine, methionine sulfoximine, and the wildfire toxin. Note that the sulfur in the first 2 compounds has been replaced by 2 carbon atoms in the toxin. One of the carbon atoms of the toxin bears the oxygen and the other the nitrogen originally attached to the sulfur of the sulfoximine. In addition, in the toxin the oxygen and nitrogen bonds have been reduced and the methyl group found at the terminal end of the first 2 compounds has been oxidized to a lactone grouping.

sulfoximine in this system.

Mutants of Chlorella selected for resistance to methionine sulfoximine were equally resistant to comparable concentrations of the wildfire toxin. The reverse was also true. One-step mutants that showed, on first plating, resistance to the highest concentrations (0.1 mg/ml) of each of the 2 compounds tested were used in both instances. This cross resistance attests further to the biological and chemical similarity of the 2 substances. Both compounds apparently interfere with the same original biosynthetic pathway. Cross resistance to the 2 compounds by mutated Chlorella cells may be due either to the ability of those cells to utilize the same alternate pathway or possibly it is due to the presence in the mutated cells of a similar type of detoxifying mechanism.

Finally, as in the case of the wildfire toxin, chlorotic lesions resulting from the application of methionine sulfoximine could not be reversed with dl- or l-methionine or any other substance or combination of substances tested. Methionine sulfoximine is clearly a structural analogue of methionine. Since many if not all of its toxic effects can be overcome in animals (6, 14) and microorganisms (3, 12) with methionine, and since this substance behaved similarly to the wildfire toxin in the various test systems reported above, the working hypothesis that the wildfire toxin, like methionine sulfoximine, is a structural analogue of methionine appeared to have been strengthened. The isolation and chemical characterization of the toxin was, however, essential for the further development of this concept. This phase of the work was brilliantly car-

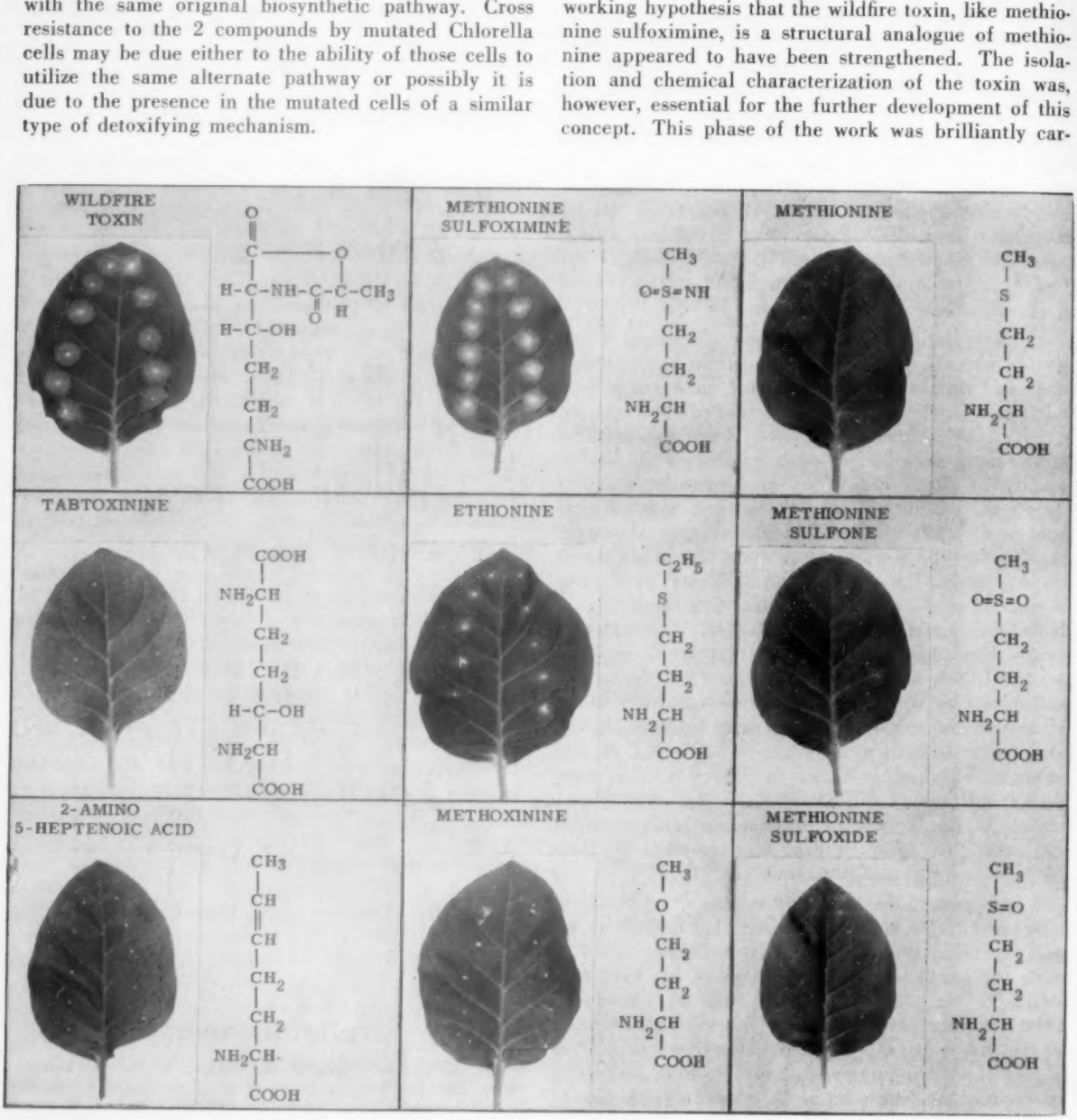



FIG. 3. A comparison of the biological activity in tobacco leaves of various structural analogues of methionine and the wildfire toxin. Of the substances tested, only methionine sulfoximine and the closely related ethionine sulfoximine reproduced toxic manifestations of the wildfire disease. In young tobacco leaves, ethionine, when used in a concentration of 1 mg/ml, caused a slight diffuse atypical yellowing around the points of inoculation, as shown above. Older leaves were unaffected by this compound. (Photographs by J. A. Carlile).

ried to fruition by Dr. D. W. Woolley despite many difficulties, not the least of which was the marked instability of the pure toxin.

The wildfire toxin has been found (19, 20) to be a derivative of a new $\alpha$-amino acid. Complete hydrolysis of the pure toxin with strong HCl yields lactic acid and large amounts of this new amino acid, which has been found to be $\alpha$, $\epsilon$-diamino-$\beta$-hydroxy pimelic acid. The name tabtoxinine has been applied to this substance. The amino acid is the second member of a new type of $\alpha$-amino acids (sulfur-free diaminodicarboxylic acid) found to occur naturally. The closely related $\alpha$, $\epsilon$-diamino pimelic acid has recently been isolated by Work (22) from hydrolysates of diphtheria and tubercle bacilli. Tabtoxinine has been found to be biologically inactive in so far as its ability either to elicit the formation of or to reverse the chlorotic effect of wildfire halos in a tobacco leaf.

The wildfire toxin has been found (21) most probably to be the lactone of $\alpha$-lactylamino-$\beta$-hydroxy-$\epsilon$-amino pimelic acid and is, as seen in Figure 4, a simple derivative of tabtoxinine.

The toxin is biologically a very active substance, and 0.05 gamma gives rise to a typical chlorotic lesion when introduced into a tobacco leaf.

That the chemically pure toxin was the native toxin and not some simple degradation product produced during the isolation procedure was demonstrated by the fact (19) that the toxin present in the crude culture filtrate had an $R_F$ corresponding to 0.26, as did the pure toxin, in an ascending paper chromatogram developed with 2:1 propanol-water. Thus, by this very mild manipulation, the isolated material could not be distinguished from the biologically active substance found in the culture fluid.

Aside from its biological activity, the most outstanding characteristic of the toxin is its marked instability. The chemically pure toxin lost activity when stored at pH 6 so that after 1 month at 0°C most of the biological activity had disappeared. The rate of destruction of the toxin increased at either side of pH 6. Approximately ½ of the activity was destroyed in 15 minutes at room temperature in 0.1 M $NaHCO_3$ at pH 8.2. The sensitivity of the toxin to alkali had previously been noted by Clayton (4). Treatment of the toxin with mild alkali gave rise to a compound which has been characterized (21) most probably as $\alpha$-lactyltabtoxinine, the structure of which is shown in Figure 4. This alkaline inactivation product resulted from the opening of the lactone ring of the toxin. When, like the toxin, the alkaline-inactivation-product was hydrolyzed by refluxing with 6N HCl, tabtoxinine was formed.

If, as has been postulated, the wildfire toxin is an analogue of methionine, it should bear a structural relationship to that essential amino acid. The relationship of methionine and the toxin can be clearly seen by comparing the structure of these compounds with that of the synthetic antimetabolite, methionine sulfoximine, the biological properties of which were considered earlier in this discussion. The formulation presented in Figure 2 illustrates this point. In the toxin the sulfur atom of both methionine and methionine sulfoximine has been replaced by 2 carbon atoms. A change such as this commonly converts metabolites into antimetabolites. One of the carbon atoms of the toxin bears the oxygen and the other bears the nitrogen originally attached to the sulfur of the sulfoximine. In addition, in the toxin, the oxygen and nitrogen

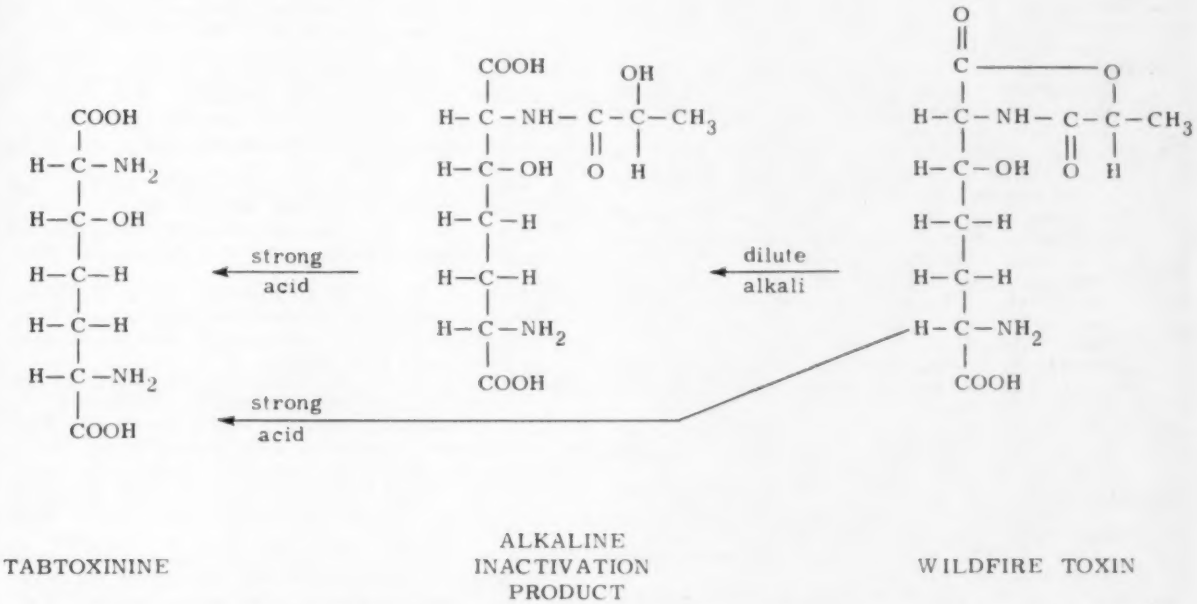



FIG. 4. The structure of compounds resulting from the treatment of the wildfire toxin with dilute alkali and strong acid. Note that dilute alkali causes the lactone ring of the toxin to open. This is accompanied by the loss of biological activity.

bonds have been reduced, and the methyl group has been oxidized to a lactone grouping.

Since the wildfire toxin and methionine sulfoximine possess biological activity when applied to a tobacco leaf whereas tabtoxinine and the alkaline-inactivation-product do not, it is interesting to speculate on the possible reason or reasons for this. It has been suggested (18) that differences in activity of these compounds may be accounted for, in part at least, by the presence or absence of ionizable groups at 1 or both ends of the molecule. The methionine molecule is ionized at only 1 end. The 2 biologically active compounds are like methionine in this respect. The lactyl group on the amino group and the lactone linkage of the carboxyl neutralize the electrical charge at the terminal end of the toxin molecule and thus make the whole toxin more closely resemble methionine. Tabtoxinine and the alkaline-inactivation-product have, on the other hand, highly ionized groups at both ends of the molecule. Thus with these 4 closely related compounds, 2 of which show biological activity whereas the other 2 do not, a correlation exists. Yet certain other structural analogues of methionine (Fig. 3) possess an ionized group at 1 end and a nonionized group at the other end of the molecule but possess no biological activity in the test systems used in these studies. It must therefore be concluded that, in addition to this characteristic, a specific but as yet uncharacterized molecular configuration common to the 2 biologically active compounds but absent in other analogues is essential for the production of chlorotic halos in a tobacco leaf. It is known from previous studies that a chemically highly reactive grouping such as the lactone grouping of the toxin may make an antimetabolite both extremely potent and irreversible in its effects. Such a reactive group presents the possibility for strongly binding the toxin by a covalent bond to the site in the plant cell that is normally occupied by methionine.

The evidence presented above indicates, then, that the wildfire toxin is a structural analogue of methionine that clearly owes its biological activity in Chlorella, and quite possibly in plants generally, to its behavior as a naturally occurring antimetabolite of that essential amino acid.

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, NEW YORK

## LITERATURE CITED

1. BENTLEY, H. R., E. E. MCDERMOTT, J. PACE, J. K. WHITEHEAD, AND T. MORAN. 1950. Toxic factor from 'agenized' proteins: methionine as the essential reactant. Nature [London] **165**: 150-151.
2. BRAUN, A. C. 1937. A comparative study of Bacterium tabacum Wolf and Foster and Bacterium angulatum Fromme and Murray. Phytopathology **27**: 283-304.
3. BRAUN, A. C. 1950. The mechanism of action of a bacterial toxin on plant cells. Nat. Acad. Sci. Proc. **36**: 423-427.
4. CLAYTON, E. E. 1934. Toxin produced by Bacterium tabacum and its relation to host range. Jour. Agr. Res. **48**: 411-426.
5. EMERSON, S. 1950. Competitive reactions and antagonisms in the biosynthesis of amino acids by Neurospora. Cold Spring Harbor Symposia on Quant. Biol. **14**: 40-47.
6. GERSHOFF, S. N., AND C. A. ELVEHJEM. 1951. The relative effect of methionine sulfoximine on different animal species. Jour. Nutr. **45**: 451-458.
7. HEATHCOTE, J. G. 1949. Toxic effect of the crystals from 'agenized' zein on certain acid-producing bacteria. Nature [London] **164**: 439.
8. HEATHCOTE, J. G., AND J. PACE. 1950. Inhibition of the growth of Leuconostoc mesenteroides by the toxic factor from 'agenized' zein: reversal by L-glutamine. Nature [London] **166**: 353-354.
9. HOROWITZ, N. H. 1947. Methionine synthesis in Neurospora. The isolation of cystathionine. Jour. Biol. Chem. **171**: 255-264.
10. JOHNSON, J., AND H. F. MURWIN. 1925. Experiments on the control of wildfire of tobacco. Wisc. Agr. Exp. Sta., Res. Bul. 62, 34 p.
11. LAMPEN, J. O., R. R. ROEPKE, AND M. J. JONES. 1947. Studies on the sulfur metabolism of Escherichia coli. III. Mutant strains of Escherichia coli unable to utilize sulfate for their complete sulfur requirements. Arch. Biochem. **13**: 55-66.
12. NEWELL, G. W., AND W. W. CARMAN. 1950. Effect of methionine on toxicity of crystalline 'agene factor' against Leuconostoc mesenteroides. Fed. Proc. **9**: 209.
13. REINER, L., F. MISANI, T. W. FAIR, P. WEISS, AND M. G. CORDASCO. 1950. Studies on nitrogen trichloride treated prolamines. IV. Isolation of the neurotoxic principle. Amer. Chem. Soc. Jour. **72**: 2297-2298.
14. REINER, L., F. MISANI, AND P. WEISS. 1950. Studies on nitrogen trichloride-treated prolamines. VI. Suppression of the development of convulsions with methionine. Arch. Biochem. **25**: 447-449.
15. SIMMONDS, S. 1948. Utilization of sulfur-containing amino acids by mutant strains of Escherichia coli. Jour. Biol. Chem. **174**: 717-722.
16. TEAS, H. J. 1950. Mutants of Bacillus subtilis that require threonine or threonine plus methionine. Jour. Bact. **59**: 93-104.
17. WOLF, F. A., AND A. C. FOSTER. 1917. Bacterial leaf spot of tobacco. Science. **46**: 361-362.
18. WOOLLEY, D. W. A naturally occurring antimetabolite of methionine in the causation of disease. Jour. Nutr. In press.
19. WOOLLEY, D. W., R. B. PRINGLE, AND A. C. BRAUN. 1952. Isolation of the phytopathogenic toxin of Pseudomonas tabaci, an antogonist of methionine. Jour. Biol. Chem. **197**: 409-417.
20. WOOLLEY, D. W., G. SCHAFFNER, AND A. C. BRAUN. 1952. Isolation and determination of structure of a new amino acid contained within the toxin of Pseudomonas tabaci. Jour. Biol. Chem. **198**: 807-813.
21. WOOLLEY, D. W., G. SCHAFFNER, AND A. C. BRAUN. 1955. Studies on the structure of the phytopathogenic toxin of Pseudomonas tabaci. Jour. Biol. Chem. **215**: 485-493.
22. WORK, E. 1951. The isolation of α, ε-diaminopimelic acid from Corynebacterium diphtheriae and Mycobacterium tuberculosis. Biochem. Jour. **49**: 17-23.

# VECTOR-FEEDING AND PLANT-TISSUE RELATIONSHIPS IN THE TRANSMISSION OF THE WISCONSIN PEA STREAK VIRUS [1]

C. B. Skotland and D. J. Hagedorn

SUMMARY

Studies of the feeding habits and plant-tissue relationships of the pea aphid, *Macrosiphum pisi* (Ketb.), were made in an attempt to determine the cause of the low efficiency of this insect as a vector of the Wisconsin pea streak virus. It was found that the pea aphid sought out the phloem to feed in and that penetration of plant tissue was primarily intercellular. This insect fed on the phloem as regularly in diseased as in healthy tissue. It rarely transmitted the virus whether having fed in phloem or in parenchyma. The data suggests that the virus is rarely transmitted by the pea aphid because this insect is largely an intercellular feeder.

Skotland and Hagedorn (8) recently reported that the pea aphid, *Macrosiphum pisi* (Ketb.), is an inefficient vector of the Wisconsin pea streak virus. This suggested that transmission of this nonpersistent virus occurs only when the vector feeds on some particular plant tissue. Fife and Frampton (2), Dykstra and Whitaker (1), and Houston et al (4), working with other vectors and viruses, have obtained evidence that certain insect-feeding and plant-tissue relationships are necessary for efficient virus transmission. This paper presents the results of studies on the feeding habits of the pea aphid in relation to transmission of the Wisconsin pea streak virus.

MATERIALS AND METHODS.—Mature apterous pea aphids were placed on pea (*Pisum sativum* L. var. Perfected Wales) plants infected with the Wisconsin pea streak virus described by Hagedorn and Walker (3). Individual aphids were given acquisition feeding periods of 1, 5, 20, and 60 minutes. The piece of tissue in which the individual aphids had fed was fixed separately in FAA (formalin-aceto-alcohol).

Upon completion of the desired acquisition feeding period, each aphid was transferred to a healthy pea plant for a 24-hour feeding period, after which the aphid was killed with an aphicide. After a suitable incubation period, the test plants upon which the supposedly viruliferous aphids had fed were examined for pea streak symptoms.

The pieces of infected tissue on which aphids that subsequently transmitted the virus to healthy plants had fed were embedded separately, and the remaining pieces of infected tissue fed upon were embedded as a group. The tissue was dehydrated in ethanol-dioxane and infiltrated and embedded in 56° Tissuemat (5). Sections were cut 15–25$\mu$ thick and stained with safranin and fast green (7). The sections were examined microscopically to determine the tissue or tissues in which the aphid had fed. This was readily discernible by studying the path of the stylet sheath (Fig. 1), which had been stained red by the safranin.

Appropriate controls were provided by feeding aphids upon healthy pea plants for the same periods as the acquisition feeding periods indicated. Tissue pieces on which they had fed were fixed and embedded, and sections were examined in a manner similar to that used for the pieces of infected tissue.

EXPERIMENTAL RESULTS.—Sheaths were found terminating 122 times in parenchyma, 152 times in phloem, and 4 times in xylem.

All 6 stylet sheaths found in tissue in which aphids had fed 1 minute were located in leaf parenchyma (Table 1) but the virus was not transmitted in any of

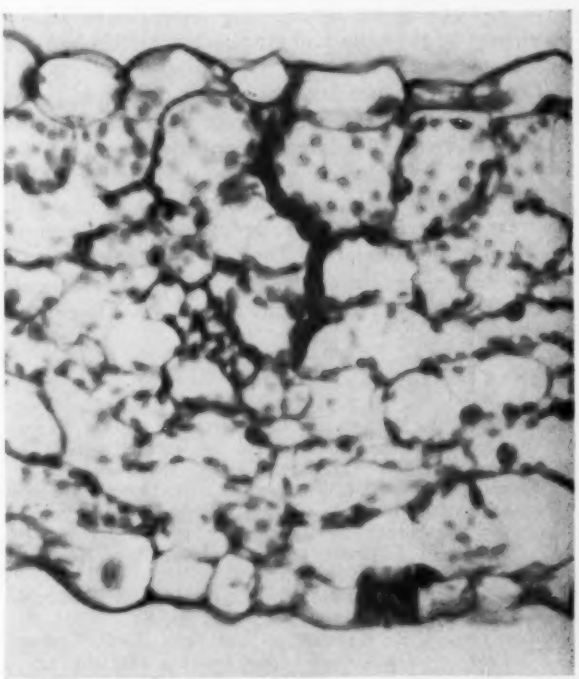


FIG. 1. Intercellular stylet sheath (dark stained line) left by the pea aphid after feeding on pea leaf. 440×.

[1] Accepted for publication July 28, 1955.

Supported in part by a grant from the Graduate School of the University of Wisconsin with funds received from the Wisconsin Alumni Research Foundation.

Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1.—*Location of stylet sheath terminus when pea aphids were fed upon healthy pea plants and upon plants infected with the Wisconsin pea streak virus*

| Acquisition feeding period (Minutes) | Condition of plant fed upon | No. of tissue pieces examined | No. of stylet sheaths found terminating in tissue indicated[a] | | | |
|---|---|---|---|---|---|---|
| | | | Parenchyma | | Phloem | |
| | | | Stem | Leaf | Stem | Leaf |
| 1 | Diseased | 35 | | 6 | | |
| 5 | Healthy | 50 | 9 | 13 | 1 | 14 |
| | Diseased | 50 | 13 | 16 | 1 | 2 |
| 20 | Healthy | 100 | 7 | 16† | 9 | 14 |
| | Diseased | 100 | 9† | 15 | 24 | 25 |
| 60 | Healthy | 100 | 6 | 2 | 22 | 7 |
| | Diseased | 100 | 3 | 7 | 15† | 18†† |

[a] Each dagger indicates 1 successful transmission of the virus.

the 35 trials.

Where 5-minute feeding periods were used, the stylet sheath was found 37 times in healthy and 32 times in diseased tissue. The aphids had fed more often in the parenchyma tissue (51 times) than in the phloem (18 times). The virus was transmitted only once—when the aphid had fed on leaf parenchyma.

When aphids were allowed a 20-minute acquisition feeding interval, they fed more commonly in phloem tissue—72 times versus 47 for parenchyma. The virus was transmitted in 1 instance when the aphid fed in stem parenchyma.

Study of sections of tissue in which aphids had fed for 60 minutes revealed a still greater contrast between phloem and parenchyma feeding in favor of the former, 62 to 18. Transmission of the Wisconsin pea streak virus was obtained 3 times—once when the aphid fed in phloem of the stem and twice when aphids had fed in leaf phloem.

The mode of penetration of the pea aphid was studied by examining stem feeding punctures made by aphids given 20 minutes' acquisition feeding. Six of the stylet sheaths were almost entirely intracellular, and 46 were intercellular. Occasionally the stylet sheath pentrated 1 or 2 cells directly and the remainder of the tissue intercellularly. Thus, it was apparent that the feeding puncture of the pea aphid was primarily intercellular. No marked differences were found between stylet paths in healthy and diseased tissue. Only rarely did the pea aphid initiate a feeding puncture through the stoma.

Examination of the 5 stylet sheaths left by aphids that subsequently transmitted the streak virus showed them to be primarily intercellular, but in each case at least 1 plant cell had been penetrated directly.

DISCUSSION.—Roberts (6) could not correlate a particular tissue fed upon by aphid vectors with transmission of any of 4 viruses studied. She reported that neither the peach aphid, *Myzus persicae* Sulz., nor the crescent-marked lily aphid, *M. circumflexus* Buckt., reached the phloem after 5 minutes' feeding, but after longer feeding intervals they were more often found feeding in that tissue. In our study, pea aphid stylets reached the phloem several times after feeding 5 minutes, and in greater numbers after 20 or 60 minutes. Even so, phloem feeding did not result in more transmissions than did parenchyma feeding.

The greatest number of transmissions was obtained with the 60-minute acquisition period. All 3 transmissions in such trials were by aphids that had fed in the phloem. In tests with diseased plants, the number of stylet sheaths in phloem tissue was greatest for the 20-minute acquisition period. Nevertheless, no transmission was obtained. These results suggest that, even though pea aphids were feeding in phloem tissue after 20 minutes, a feeding interval longer than 20 minutes is necessary before even small numbers became viruliferous.

The transmission of the Wisconsin pea streak virus could not be correlated with mode of penetration of the pea aphid stylet because only 1 virus transmission was achieved in such studies. The pea aphid stylet sheath was primarily intercellular. In contrast, all 3 stylet sheaths left by aphids given 60-minute feeding periods and successfully transmitting the virus were found to be at least partly intracellular. This suggests that intracellular stylet penetration is conducive to virus transfer and that the pea aphid is an inefficient vector of Wisconsin pea streak virus because its feeding is primarily intercellular.

DEPARTMENTS OF PLANT PATHOLOGY AND AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

LITERATURE CITED

1. DYKSTRA, T. P., AND W. C. WHITAKER. 1938. Experiments on the transmission of potato viruses by vectors. Jour. Agr. Res. **57**: 319-334.
2. FIFE, J. M., AND V. L. FRAMPTON. 1936. The pH gradient extending from the phloem into the parenchyma of the sugarbeet and its relation to the feeding behavior of Euttettix tenellus. Jour. Agr. Res. **53**: 581-593.
3. HAGEDORN, D. J., AND J. C. WALKER. 1949. Wisconsin pea streak. Phytopathology **39**: 837-847.
4. HOUSTON, B. R., KATHERINE ESAU, AND W. D. HEWETT. 1947. The mode of vector feeding and the tissue involved in the transmission of Pierce's disease virus in grape and alfalfa. Phytopathology **47**: 247-253.
5. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
6. ROBERTS, F. M. 1940. Studies on the feeding methods and penetration rates of Myzus persicae Sulz., Myzus circumflexus Buckt., and Macrosiphum gei Kock. Ann. Appl. Biol. **27**: 348-358.
7. SASS, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill, New York. 222 p.
8. SKOTLAND, C. B., AND D. J. HAGEDORN. 1954. Aphid transmission of the Wisconsin pea streak virus. Phytopathology **44**: 569-571.

# PHYSIOLOGY AND NATURE OF DISEASE DEVELOPMENT IN WINTER CROWN ROT OF ALFALFA [1]

J. B. Lebeau [2] and J. G. Dickson [3]

SUMMARY

The development of winter crown rot of alfalfa is associated with absorption by the crown tissue of lethal concentrations of HCN that is produced by an unidentified low-temperature basidiomycete. Under controlled conditions, both dormant and active alfalfa plants were killed, and concentrations of as much as 2400 ppm HCN were recovered from the diseased tissues. Factors that increased the production of HCN and its retention in the crown area of the plant were essential for severe disease development. Maximum HCN synthesis in culture occurred at 12°C, and organic sources of nitrogen were more favorable for synthesis than were inorganic ones. Mycelium grew more rapidly on asparagine and sucrose than on media containing other nitrogen and carbon sources. The fungus entered the host at the apex or base of the crown bud and advanced to the crown and shoot tissues through the vascular system. No differential or defensive response to the pathogen was observed in the host tissue.

Many of the causes of winter injury of alfalfa plants are not clearly understood. The various factors essential for the development of winter crown rot (5) as distinct from cold injury, have been difficult to ascertain. Previous investigations of this disease have been concerned largely with its distribution and development under field conditions (3, 4, 5, 6). In the present study, the investigations of 1 of the pathogens associated with winter crown rot, namely, the low-temperature basidiomycete, were directed initially toward the physiology of the fungus. Several reasons for the initial study of the fungus metabolism were obvious following a review of the earlier investigations on this specific disease. The pathogen apparently persisted as mycelium with clamp connections in both the natural environment and in culture; therefore, it was impossible to identify it except as a basidiomycete. Information on the histology and physiology of pathogenicity was inadequate, although the association of the fungus with the syndrome of the disease had been established. Finally, the unusual capacity of this fungus to grow at temperatures near 0°C (3) and to produce HCN during mycelial growth (9) presented a challenge to investigate the physiology of metabolism. The present paper is a survey of some aspects of the problem.

PHYSIOLOGY OF THE FUNGUS.—*The effect of some carbohydrate and nitrogen sources on dry weight of mycelium.*—The influence of temperature on the ability of the fungus to utilize a few representive monosaccharides, disaccharides, polysaccharides, inorganic and organic nitrogen sources, and other plant substances was investigated as a possible means of determining the activity of some of the enzymes present in the fungus and the general type of metabolism.

The fungus (B-24) was obtained from diseased alfalfa plants at Edmonton, Alberta. Growth rates of the mycelium were determined on 40 ml of autoclaved liquid nutrient media in 125-ml Erlenmeyer flasks. The cooled medium was seeded with 7-mm agar plugs of the mycelium. After 35 days' incubation at 2°, 4°, and 8°C, the mycelium was removed, dried to a constant weight, and weighed.

For the synthetic media, a basal Richards' solution (containing $KH_2PO_4$, 5 g; $MgSO_4$, 2.5 g; $FeCl_3$, 0.2 g; distilled $H_2O$ to 1 liter) was used. Four different carbon and 2 different nitrogen sources were added to this basal solution for test, namely, glucose (50 g/l), sucrose (42 g/l), xylan and pectin (each 12.5 g/l), $KNO_3$ (10 g/l) and asparagine (5.2 g/l).

For media from natural sources, crown tissue of susceptible *Medicago sativa* L. and of the resistant *M. falcata* L. (Alaskan strain) were oven-dried at 85°C, and each was ground to a fine meal in a Wiley mill. One-gram portions were placed in pieces of glass tubing 2 in. long and 10 mm in outside diameter, and both ends were plugged loosely with glass wool. One such tube was placed in 40 ml of distilled water in each of the flasks used with the plant materials. The same type of tube was used to suspend xylan in the basic nutrient solution. The flasks containing the tubes and water or basic mineral nutrient solution were then autoclaved, cooled, and seeded with the fungus. Four replicates were used for each experiment and each experiment was repeated once. Determinations were made of the average dry weights of the mycelial mats,

[1] Accepted for publication July 27, 1955.

Joint contribution from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario (Contribution No. 1472) and the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin. From a thesis presented by the senior author to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Acknowledgment is made to Dr. Réal Pelletier for the advice and assistance in first detecting the presence of HCN in cultures; to Dr. Dale Smith and Dr. F. R. Jones for counsel and for plants; to Dr. G. B. Sanford for his interest and for plant materials; and to Eugene Herrling for preparing figures and photographs.

[2] Associate Plant Pathologist, Plant Pathology Laboratory, Edmonton, Alberta.

[3] Professor of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

the pH of the solutions after autoclaving, and the final pH after the fungus mat was removed.

The fungus cultures were incubated in the dark in constant-temperature chambers. The average dry weight of mycelium after 35 days and the drift in pH for 3 carbon sources with asparagine and alfalfa crown tissue are given in Table 1. One series with potassium nitrate and glucose was included. Pectin-asparagine medium produced the highest weights of mycelium with a rise in pH relatively proportional to the mycelial weight. Alfalfa meal, xylan, and glucose were utilized in descending order on the basis of weight of mycelium. The optimum temperature for the production of dry weight of mycelium in 35 days was around 12°C on most of the media with a very high growth rate at as low as 4°.

The influence of 4 sources of carbon upon dry weight of mycelium are compared with asparagine and potassium nitrate as nitrogen sources at 2°, 4°, and 8°C (Fig. 1). In 1 series the solutions were buffered to approximately pH 6 by the addition of 2 ml of McIlvaine's buffer solution per flask (7). In the other series the pH was not adjusted. Both glucose and sucrose in combination with asparagine were superior to pectin as a carbon source in the buffered solutions. In the unadjusted solutions, pectin produced higher weights than glucose as in the previous experiments. The mycelial growth with sucrose in the unbuffered solutions was superior to that obtained when pectin and glucose were employed at the higher temperature. Potassium nitrate was inferior as a nitrogen source in all comparisons, although the response with pectin in the buffered solutions was not greatly different from that of asparagine with the same carbon source.

Preliminary results with other sources of nitrogen and pectin as the carbon source are given in Table 2. Ammonium sulphate at 8°C was equivalent to asparagine as a source of nitrogen. Casein hydrolysate reduced growth below that obtained with the individual amides, and yeast extract apparently exerted a growth-suppressing effect.

The mycelial weights obtained with alfalfa crown tissue as the sole source of nutrients are given in Table 3. Growth of the fungus at 4° and 8° with crown tissues of the 2 species was similar. The natural buffering capacity of the crown tissues maintained a constant pH during the period of the experiment.

The slightly lower pH of the crown tissue of the

TABLE 1.—*Average dry weight of mycelium and drift in pH of the culture solutions containing different carbon and nitrogen sources at temperatures from 2°–24°C*

| Medium | Temperature | Initial pH | Final pH | Weight of mycelium |
|---|---|---|---|---|
| | °C | | | mg |
| Basal medium[a] + asparagine and pectin | 2 | 3.8 | 3.8 | 27.7 |
| | 4 | 3.8 | 4.0 | 158.6 |
| | 8 | 3.8 | 5.2 | 172.2 |
| | 12 | 3.8 | 6.2 | 228.5 |
| | 16 | 3.8 | 6.3 | 227.4 |
| | 20 | 3.8 | 6.4 | 217.4 |
| | 24 | 3.8 | 3.5 | 0.0 |
| Basal medium + asparagine and cerelose | 2 | 4.2 | 4.1 | 51.7 |
| | 4 | 4.3 | 4.3 | 82.4 |
| | 8 | 4.1 | 4.1 | 89.1 |
| | 12 | 4.2 | 4.0 | 96.7 |
| | 16 | 4.2 | 4.1 | 88.2 |
| | 20 | 4.3 | 4.1 | 77.0 |
| | 24 | 4.3 | 4.3 | 0.0 |
| Basal medium + potassium nitrate and cerelose | 2 | 4.0 | 4.0 | 12.1 |
| | 4 | 3.9 | 4.0 | 25.1 |
| | 8 | 4.1 | 3.9 | 32.6 |
| | 12 | 4.0 | 3.7 | 44.9 |
| | 16 | 4.0 | 3.6 | 56.9 |
| | 20 | 4.0 | 3.7 | 9.5 |
| | 24 | 4.0 | 4.0 | 0.0 |
| Basal medium + asparagine and xylan | 2 | 4.9 | 4.6 | 45.8 |
| | 4 | 5.0 | 5.4 | 94.7 |
| | 8 | 5.1 | 5.3 | 127.1 |
| | 12 | 5.1 | 5.1 | 125.6 |
| | 16 | 5.1 | 5.1 | 81.2 |
| | 20 | 5.0 | 4.9 | 54.6 |
| Alfalfa crown tissue | 2 | 6.0 | 5.7 | 7.4 |
| | 4 | 5.9 | 6.0 | 127.2 |
| | 8 | 5.9 | 5.8 | 177.0 |
| | 12 | 5.9 | 5.9 | 182.9 |
| | 16 | 5.8 | 5.8 | 164.7 |
| | 20 | 5.9 | 5.8 | 160.7 |
| | 24 | 6.0 | 5.8 | 0.0 |

[a] Richards' solution.

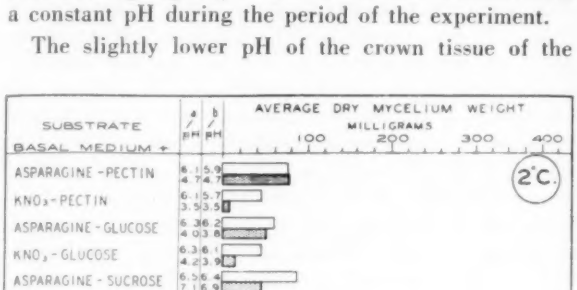



FIG. 1. The effect of different carbon and nitrogen sources, at 2°, 4°, and 8°C, on the growth rate of the low-temperature basidiomycete.

TABLE 2.—*Growth of the low-temperature basidiomycete at 8°C on media containing different nitrogen sources and yeast extract*

| Substrate | Initial pH | Final pH | Ave. weight of mycelium |
|---|---|---|---|
| | | | mg |
| Basal medium[a] with | | | |
| Glutamine | 5.7 | 5.9 | 233 |
| Asparagine | 5.6 | 6.3 | 201 |
| $(NH_4)_2SO_4$ | 5.6 | 5.7 | 200 |
| Casein hydrolysate | 6.2 | 5.5 | 158 |
| $KNO_3$ | 5.6 | 5.7 | 152 |
| Asparagine + yeast extract[b] | 6.1 | 6.2 | 132 |

[a] Each nitrogen source with equivalent concentrations of nitrogen was added to a basal solution containing Richards' solution and pectin.
[b] 0.1 Per cent yeast extract added.

resistant *Medicago falcata* compared with the susceptible *M. sativa* was consistent, although the difference in dry weights of fungus produced on the 2 substrates was not significant.

*The effect of nutrient media on the production of HCN.*—During the study of the effect of media on the growth of the fungus at low temperatures, the evolution of HCN was detected in flasks containing the fungus growing on a soil-soybean medium. The fungus had produced large mats of mycelium on this medium during a period of 60 days at 4°C. HCN concentration in the flasks was high (9). Further experiments with other media demonstrated that the production of HCN during the growth of the fungus was a characteristic phenomenon.

The fungus was cultured on synthetic and natural media as described previously and grown at 4°, 8°, 12°, and 20°C. Flasks were removed from the series and HCN was determined every 10 days.

The method used for determining the HCN was essentially that outlined by Boyd (2) in connection with Sudan grass. The samples were steam-distilled into a 2 per cent solution of KOH; then 5 ml of the distillate was added to the solution of sodium picrate (yellow), which is reduced by HCN to sodium picramate (red). The HCN concentrations were determined with the Evelyn photoelectric colorimeter by comparison with standard solutions of known HCN concentration. Control determinations were made on noninoculated samples to detect whether or not steam-volatile reducing substances other than HCN might be present. The concentration of HCN was expressed as ppm of the original dry weight of nutrients or plant tissues.

TABLE 3.—*Average dry weights of mycelium of the low-temperature basidiomycete when grown on alfalfa crown tissue for a period of 35 days*

| Plant used for medium | Stage of plant growth | Temperature | Initial pH | Final pH | Dry weight of mycelium |
|---|---|---|---|---|---|
| | | °C | | | mg |
| *Medicago sativa*[a] | Dormant | 2 | 6.4 | 6.2 | 72 |
| *M. sativa*[a] | Active | 2 | 6.3 | 6.2 | 97 |
| *M. falcata*[a] | Dormant | 2 | 5.2 | 5.2 | 43 |
| *M. sativa*[a] | Dormant | 4 | 6.2 | 6.2 | 141 |
| *M. sativa*[a] | Active | 4 | 6.1 | 6.0 | 117 |
| *M. falcata*[a] | Dormant | 4 | 5.9 | 5.8 | 141 |
| *M. sativa*[a] | Dormant | 8 | 6.0 | 6.0 | 168 |
| *M. sativa*[a] | Active | 8 | 6.1 | 6.2 | 158 |
| *M. falcata*[a] | Dormant | 8 | 5.8 | 5.8 | 147 |
| *M. sativa*[b] | Dormant | 2 | 6.5 | 6.8 | 33 |
| *M. sativa*[b] | Active | 2 | 6.4 | 6.4 | 31 |

[a] Dry ground tissue sterilized in autoclave.
[b] Fresh wet tissue sterilized with propylene oxide.

The average production of HCN by the fungus grown on alfalfa-crown meal for 30 days at different temperatures is shown graphically in Figure 2, A. The rate of HCN production of the fungus at 12° on a substrate of soybean meal is shown in Figure 2, B. The data in Table 1 and Figure 2, A suggest that the optimum temperature for both HCN production and mycelial growth was about 12° on alfalfa crown meal.

PATHOGENICITY OF THE FUNGUS.—The pathogenicity of the fungus was determined under conditions favorable for the direct penetration and establishment of the fungus in the crown tissues of alfalfa (*M. sativa*) and under conditions favorable for the accumulation of HCN around the crowns. Both dormant and growing plants were studied under several environments.

The plants were produced from seed sown in the field at Madison, Wisconsin, in May of each year. Uniform plants were removed from the field at intervals from September to April. Plants taken from the field in the autumn before November were vegetatively active and free from winter injury. Plants removed in April were starting new growth following the winter dormancy. The growing plants were washed free of soil and used immediately in the inoculation experiments or potted and placed in the greenhouse or cold frames for later use. Dormant plants removed from the field after November were washed free of soil and used directly or wrapped in moist packing and stored at −2°C until used. Inoculation experiments were conducted on plants placed in soil in the greenhouse or on plants placed in water in Mason jars in the temperature-controlled incubators. Cyanide determinations were made on plants selected at random at the end of the experiments. The plant tissues were washed in cold water and placed immediately in the distillation flask. HCN concentrations are expressed on a basis of the dry weight of the crown tissues.

The first greenhouse experiment was designed to reproduce the disease in actively growing plants. The alfalfa plants were potted in a silt soil with alfalfa crown meal inoculum placed around the crowns of each plant, and the surface of the soil was then covered with a thin layer of moist expanded mica. The controls consisted of an equal number of noninoculated plants. The pots were held for a short time at 8°C, then the soil temperature was gradually decreased to 2° and held there for a period of 50 days. The stems of the plants extending above the soil surface developed at 16°. The plants showed some vegetative activity under these conditions as growth of the

shoots was evident over the 50-day period. Sixty-seven per cent of the plants in the inoculated series were dead in contrast to none in the control. No data on the absorption of HCN by the diseased plants were obtained because its presence was not suspected until after the conclusion of this experiment.

In a further experiment, the thin covering of expanded mica used in the first trial was replaced by nonabsorbent cotton, hermetically sealed on top by paraffin wax having a low melting-point. Also, the inoculum was mixed in the upper 2 in. of soil in the pots instead of being placed about the crowns as in the first test, and the incubation was continued for 14 days at a soil temperature of 4°. Under these conditions, only 1 per cent of the inoculated plants survived and 90 per cent of the controls remained healthy. The average concentration of HCN recovered from the crown tissue of the inoculated plants was 1400 ppm.

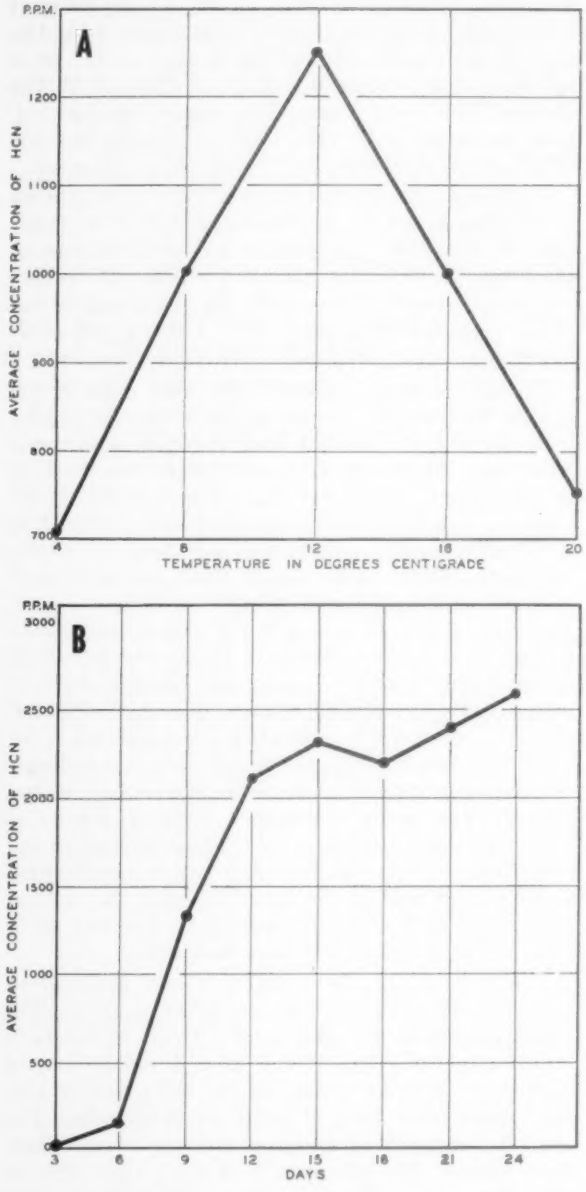



FIG. 2. The effect of time and temperature on synthesis of HCN by the low-temperature basidiomycete. A) Synthesis of HCN at end of a 30-day period on a substrate of alfalfa crown meal, at temperatures of 4°, 8°, 12°, 16°, and 20°C. B) Synthesis of HCN at 12°C on a substrate of soybean meal as determined at 3-day intervals during 24 days.

Disease development in dormant plants at temperatures closer to the optimum for mycelial growth was studied in the greenhouse. The soil was held at temperatures between 8° and 12°C for 60 days. Inoculum consisting of ground alfalfa crown tissue infested with the fungus was mixed in the upper 2 in. of soil. The surface of the pots was covered with moist expanded mica. No disease developed under these conditions, despite the fact that the mycelium grew well, and no HCN was absorbed by the crown tissues of the experimental alfalfa plants grown at these high temperatures.

The relative effect of the fungus on dormant alfalfa plants when inoculated with the fungus growing on 4 different substrates was evaluated in an incubator held at 4°C. The substrates were potato-cerelose agar; 9 parts of soil mixed in 1 part of cornmeal; 9 parts of soil and 1 part of alfalfa crown meal; and 9 parts of soil and 1 part of soybean meal.

The inoculum (substrate plus mycelium) was placed inside moist nonabsorbent cotton and fastened around the crown of each plant. The plants were placed in 1-pint Mason jars, with their roots in about 2 in. of distilled water. The controls were treated in the same manner; the substrate with fungus omitted was placed around the crowns. The sealed jars were placed in an incubator for 50 days at a temperature of 4°, since preliminary experiments had indicated that the greatest damage to alfalfa plants occurred at this combination of time and temperature. The cyanide recovered varied from 134 ppm obtained in the crown tissues that had been inoculated with the fungus growing on potato-cerelose agar to 2400 ppm found in tissues inoculated with the fungus in the mixture of soil and alfalfa crown meal (Table 4). Less mycelium developed on the potato-cerelose agar than on the other 3 substrates. The composition of the substrate in the absence of the fungus apparently affected the survival of the plants in the control series.

TABLE 4.—*Average HCN recovered from diseased crowns of alfalfa plants held at 4°C for a period of 50 days*

| Inoculum | Survival of plants | HCN in crown tissue |
|---|---|---|
| | per cent | ppm |
| Soil plus cornmeal | 0 | 640 |
| None | 75 | nil |
| Soil plus alfalfa crown meal | 0 | 2400 |
| None | 85 | nil |
| Soil plus soybean meal | 0 | 660 |
| None | 60 | nil |
| Potato-cerelose agar | 10 | 134 |
| None | 100 | nil |

PATHOLOGICAL HISTOLOGY.—Cormack (5) noted that alfalfa plants were killed or greatly weakened in the field when only the crown buds and a very small portion of the underlying crown tissue were affected. In the present investigation, the mode of penetration of the host by the fungus and the tissues affected were investigated under conditions favorable for HCN accumulation.

The crown buds and shoots were removed from plants in which disease developed at controlled temperatures of 2°–4°C. HCN determinations were made of these tissues, and sections were cut at regular intervals during 60 days of incubation. The plant parts were fixed for at least 7 days in FAA solution (90 parts 70 per cent ethyl alcohol, 5 parts 40 per cent formalin, and 5 parts glacial acetic acid) then dehydrated in ethyl alcohol, dioxan, and tertiary butyl alcohol. The tissue was then transferred to 50 per cent paraffin and 50 per cent tertiary butyl alcohol, and finally to 100 per cent paraffin. Three days later paraffin with a melting point of 50°C was added. Three changes of paraffin were made before the material was cast into blocks. The embedded crown-bud tissue was cut about 12$\mu$ thick and the crown shoots about 15$\mu$. Conant's quadruple stain (8) was used to obtain a good contrast between the mycelium and host tissue. The fungus was identified by the typical clamp connections (Fig. 3, A).

Examination of the stained sections showed that during the first 30 days after inoculation, the fungus remained outside the plant tissue. In some sections, masses of the mycelium were found between some of the outer bud scales. Mass invasion of the host tissue by the pathogen occurred 45–60 days after inoculation (Fig. 3, B). The fungus entered the host tissues either at the apex or base of the crown bud. Large aggregates of mycelium formed beneath the bud scales and invaded the parenchymatous tissue and later the vascular elements of the bud scales. The mycelium advanced through the vascular tissues to the stem and crown tissues. In nearly all cases, growth of the mycelium between the parenchymatous cells throughout the bud and shoot tissue was parallel to the long axis of the tissue. The mycelium was both intercellular and intracellular (Fig. 3. C, D, E). The development of the mycelium through the host cell walls occurred without the formation of special structures of penetration. After entering the host cell, the fungus usually filled the entire lumen with masses of mycelium (Fig. 3, E). The host tissue appeared to remain intact, and cellular reactions between the host and the pathogen were not evident. Also, no defensive response of the cells of the cambium or parenchyma of the host tissue was evident (Fig. 3, F).

Cyanide determinations made on these tissues 45–60 days after inoculation showed concentrations upwards of 2000 ppm. This would indicate that these cells were dead before the time of sectioning.

DISCUSSION.—Previously, the development of winter crown rot of alfalfa has been thought to be related to the physiological changes accompanying dormancy of the host plant. On the basis of experimental data, Cormack (5) showed that a definite period of association between the pathogen and the host was required in the fall and early winter before the host became susceptible to the disease, and that the dormant plants appeared to become progressively more susceptible.

The evidence presented in this paper indicated that a rather long period of 45–60 days of association between host and pathogen was required for the absorption of lethal concentrations of cyanide by the host. Dormant plants may be more susceptible to injury from cyanide than vegetatively active tissues, but the results from the present study do not indicate significant differences. Growing plants were killed in 14 days by the low-temperature basidiomycete when conditions were favorable for maximum production and retention of HCN in the crown area of the plants.

Histological evidence showed that mass invasion of the host had not occurred until the tissues had absorbed lethal quantities of HCN. This suggested a low form of parasitism with the greater development of the organism on the dead tissues of the host.

The response in mycelial growth and HCN synthesis to various types of media indicates that this pathogen can utilize a wide range of nutrients at temperatures far below its optimum for growth and in some cases at a pH of 3.5. Nitrogen of glutamine and asparagine was utilized more readily than $KNO_3$; however, $(NH_4)_2SO_4$ gave a greater growth response than casein hydrolysate. Water extracts from both resistant and susceptible plants were equally suitable as substrates for the fungus. In view of these data it is unlikely that nutritional differences in the living hosts are important in the development of this disease, although the evidence suggests that differences in quantity of HCN produced were associated with nutrient media.

The maximum synthesis of HCN by the pathogen cultured on alfalfa crown meal occurred at about 12°C. A lower temperature (2°–4°), however, was essential in all experiments for disease expression. The relation of low temperature to cyanide injury has been reported by Quale and Rohrbaugh (10), who found that rooted lemon cuttings, preconditioned and fumigated with HCN at 50°F, were more severely injured by the cyanide than those treated at 90°F. Bartholomew et al (1) found that in citrus fumigation the recovery of HCN from fruits preconditioned at 43°F was approximately twice that recovered from fruits preconditioned at 80°F. They explained this relationship of temperature to HCN absorption in fruits on the basis of the gas law which states that the lower the temperature the greater the solubility of a gas in a solvent.

The high concentrations of HCN (up to 2400 ppm on a dry weight basis) recovered in the present studies from diseased alfalfa crowns inoculated and incubated at 2°–4°C may help to explain the pathogenicity of the low-temperature basidiomycete in the

FIG. 3. Mycelium of low temperature basidiomycete in young host tissue of alfalfa, with time in days after inoculation indicated and at an incubation temperature of 2°C. A) Mycelium in pith of shoot showing clamp connections, 60 days (×1350). B) Progress of mycelium from apex of crown downward in crown bud scale, 60 days (×70.5). C) Intercellular (×200) and D) intracellular (×600) ramification of fungus in cortical cells after 45 days. E) Ramification of fungus after 60 days in vascular cells of young shoot (×1350). F) Extensive invasion of cambial region (×1350).

field at temperatures much below its optimum for growth. The production of HCN by this basidiomycete is associated logically with the etiology of this disease. Winter crown rot flourishes at low temperatures in a surface-frozen soil, a condition that tends to prevent the gas from escaping and results in injury to the crown bud tissues.

PLANT PATHOLOGY LABORATORY
EDMONTON, ALBERTA, CANADA

LITERATURE CITED

1. BARTHOLOMEW, E. T., W. B. SINCLAIR, AND D. L. LINDGREN. 1942. Measurements on hydrocyanic acid absorbed by citrus tissues during fumigation. Hilgardia **140**: 373-409.
2. BOYD, F. T. 1935. The determination of factors influencing the amount of cyanide in Sudan grass. Ph.D. Thesis, University of Wisconsin, Madison, Wisconsin.
3. BROADFOOT, W. C., AND M. W. CORMACK. 1941. A low-temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopathology **40**: 847-859.
4. CORMACK, M. W. 1942. Varietal resistance of alfalfa and sweet clover to root and crown rotting fungi in Alberta. Sci. Agr. **22**: 775-786.
5. CORMACK, M. W. 1948 . Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. 1. Occurrence, parasitism, and spread of the pathogen. Canad. Jour. Res., Sect. C, **26**: 71-85.
6. CORMACK, M. W. 1952. Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. II. Field studies on host and varietal resistance and other factors related to control. Canad. Jour. Bot. **30**: 537-540.
7. HODGMAN, C. D., ed. 1948. Handbook of chemistry and physics. Ed. 30. Chemical Rubber Publ. Co. Cleveland, Ohio. 2686 p.
8. JOHANSEN, D. A. 1940. Plant microtechnique. Ed. 3. McGraw-Hill, New York. 523 p.
9. LEBEAU, J. B., AND J. G. DICKSON. 1953. Preliminary report on production of hydrogen cyanide by a snow mold pathogen. Phytopathology **43**: 581-582.
10. QUALE, H. J., AND P. W. ROHRBAUGH. 1934. Temperature and humidity in relation to HCN fumigation for red scale. Jour. Econ. Entom. **27**: 1083-1095.

# EFFECT OF BRIEF EXPOSURES AT 40°C ON GERMINATION OF SPORANGIA OF PHYTOPHTHORA INFESTANS [1]

Carlton F. Taylor [2], John J. Smoot, D. O. Quinn[3], R. A. Rohde[4], and Edward S. Elliott [5]

SUMMARY

Exposure of young sporangia of *Phytophthora infestans* to 40°C for from 1 to 20 minutes accelerated the rate of direct germination on agar, although it induced a lag in the start of germination. The longer the period of exposure, the longer was the lag that followed. Longer exposures reduced total germination. Indirect germination in water was reduced markedly by an exposure as brief as 1 minute. There was little germination following exposures longer than 5 minutes.

The effect of pregermination exposures of fungus spores to high temperatures has received little attention from previous workers. Dodge[6] and Goddard[7], working with dormant ascospores of Ascobolus and Neurospora, found that brief exposures at high temperature were effective in inducing germination. Such observations have not been extended to asexual spores from young cultures where dormancy would not be expected to be a factor. In the present study, sporangia of *Phytophthora infestans* (Mont.) d By. from young cultures on agar were exposed to 40°C for different periods of time with marked effect on the rate and time of germination.

METHODS.—Ten- to 14-day-old cultures on lima bean agar were washed with distilled water. The washings were passed through a fine wire screen to remove mycelial mats, and the sporangia were concentrated by centrifugation. Twenty ml of distilled water was heated to 40°C in a water bath, and 1 ml of the concentrate was added to the heated water. The suspension was kept in the water bath where the temperature of the suspension quickly returned to 40° after a brief initial drop of approximately 0.5°. The suspension was shaken, and the samples were withdrawn at desired intervals.

[1] Accepted for publication August 9, 1955.
[2] Extension Plant Pathologist, West Virginia University Experiment Farm, Kearneysville, West Virginia.
[3] Graduate Assistant, Department of Plant Pathology, West Virginia University.
[4] Graduate Assistant, Department of Botany, University of Maryland.
[5] Assistant Professor of Plant Pathology, West Virginia University.
[6] Dodge, B. O. 1912. Methods of culture and morphology of the archicarp in certain species of the Ascobolaceae. Torrey Bot. Club Bul. 49: 139-197.
[7] Goddard, D. R. 1935. The reversible heat activation inducing germination and increased respiration in the ascospores of Neurospora tetrasperma. Jour. Gen. Physiol. 19: 45-60.

TABLE 1.—*Direct germination of heat-treated sporangia at varying periods of incubation at 20°C*

| Minutes at 40°C. | Percentage[a] of sporangia germinated after indicated incubation periods (hours) 4 | 6 | 8 | 10 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 48 | 56 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | t | 4 | 6 | 6 | 9 | 13 | 15 | 22 | 29 | | 41 | 51 | 52 |
| 1 | | 5 | 30 | 38 | 39 | 36 | 39 | 41 | 45 | | 47 | 49 | |
| 2.5 | | t | 23 | 37 | 46 | 50 | 45 | 47 | 52 | | 53 | | |
| 5 | | | 4 | 25 | 49 | 48 | 53 | 57 | 58 | | 61 | | |
| 10 | | | t | 2 | 11 | 45 | 50 | 57 | 60 | | 63 | | |
| 15 | | | | t | 2 | 12 | 33 | 48 | 54 | 50 | 57 | 53 | |
| 20 | | | | | | 4 | 13 | 36 | 49 | | 50 | 48 | |
| 30 | | | | | | | 2 | 9 | 12 | 15 | 22 | 23 | 23 |
| 40 | | | | | | | t | 1 | 3 | | 8 | 7 | 10 |
| 50 | | | | | | | | | | | 1 | 1 | 2 |
| 60 | | | | | | | | | | | | t | |

[a] Each figure is based on 600 sporangia. The letter t indicates less than 0.5 per cent germination.

*Direct germination.*—Samples were sprayed on the surface of 2 per cent peanut-hull-extract agar[8] in Syracuse dishes and incubated at 20°C. Direct germination was determined by counting the percentage of sporangia that had produced germ tubes. Depending upon the purpose of the experiment, counts were made at frequent intervals on the same dishes, or a single count was made after 18–20 hours. Germination was stopped when desired by placing a small drop of formalin at the inside edge of the dish.

*Indirect germination.*— Samples were pipetted to Syracuse dishes containing a small amount of distilled water and the dishes incubated at 10°C. Counts were made of the percentage of spores from which the contents had escaped as zoospores. As with direct germination, counts were made in the same dishes at intervals, or germination was stopped when desired by adding a drop of formalin. Interval counts on the same dishes introduced a slight unavoidable variation in temperature in that dishes were exposed to an increased temperature during counting periods. When necessary, both indirect and direct germination were counted.

RESULTS.—*Direct germination.*—When the counts were made at 20 hours, the germination followed the curve shown in Figure 1. The unheated control lots showed a germination of only 15 per cent, less than that following heat treatment of as long as 15 minutes. The highest germination resulted from an exposure of 5 minutes. With longer exposures the germination rate decreased rapidly. These data are typical of several similar experiments conducted. While the maximum germination attained varied with the different lots of sporangia, the pattern of stimulation following treatment was consistent.

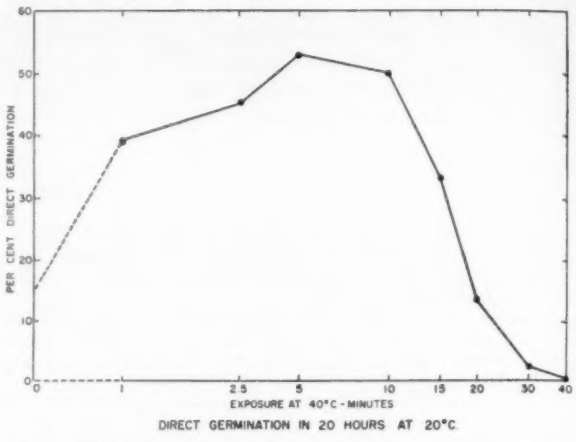



FIG. 1. Effect of the duration of exposure of sporangia at 40°C on amount of direct germination after 20 hours, expressed as percentage of 600 sporangia.

When counts were made at intervals during the germination process, the effects of the heat treatment were found to be even more striking (Table 1 and Fig. 2). The rate of germination of the controls was approximately linear, starting at 4 hours and continuing at a fairly uniform rate for 48 hours. In contrast, heat treatments up to 20 minutes progressively delayed onset of germination. Once started, however, germination progressed at a very rapid rate. With exposures greater than 20 minutes, the final germination was progressively reduced until less than 1 per cent of the sporangia were viable after a 60-minute exposure. With these longer exposures, the germination rate failed to follow the sigmoid curve typical of the shorter exposures.

*Indirect germination.*—At 10°, indirect germination

[8] Snieszko, S. F., J. B. Carpenter, E. P. Lowe, and J. G. Jakob. 1947. Improved methods for the cultivation and storage of Phytophthora infestans. Phytopathology 37: 635-649.

TABLE 2.—*Indirect and direct germination of heat-treated sporangia at varying periods of incubation at 10°C*

| Minutes at 40°C | Percentage germination[a] after indicated incubation periods (hours) 1 | 2 | 4 | 6 | 8 | 10 | 12 | 16[b] | 20[b] | 24 | 28 | 32 | 36 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 22 | 39 | 52 | 70 | 73 | 73(t) | 80(1) | 73 | 81(2) | 89(2) | 85(1) | | | 80(1) |
| 1 | | | 3 | 6 | 12 | 12 | 25 | 17 | 30(1) | 38(3) | 38(3) | | | 46(7) |
| 2.5 | | | | 1 | 3 | 6(t) | 21(1) | 5 | 3(1) | 26(11) | 28(12) | | 42(14) | 42(17) |
| 5 | | | | | t | 2 | 3 | 2 | 1 | 9(2) | 8(10) | 12(39) | 24(31) | 18(39) |
| 10 | | | | | | | 1 | 1 | 1 | 1(t) | 1(1) | | | 3(5) |

[a] Figures in parenthesis indicate direct germination, others indicate indirect germination. Each figure is based on 600 sporangia. The letter t indicates less than 0.5 per cent germination.

[b] Each count in these columns was made on a separate dish in which the sporangia were killed with formalin at the end of the incubation period. Other counts in a given row were made on the same dish.

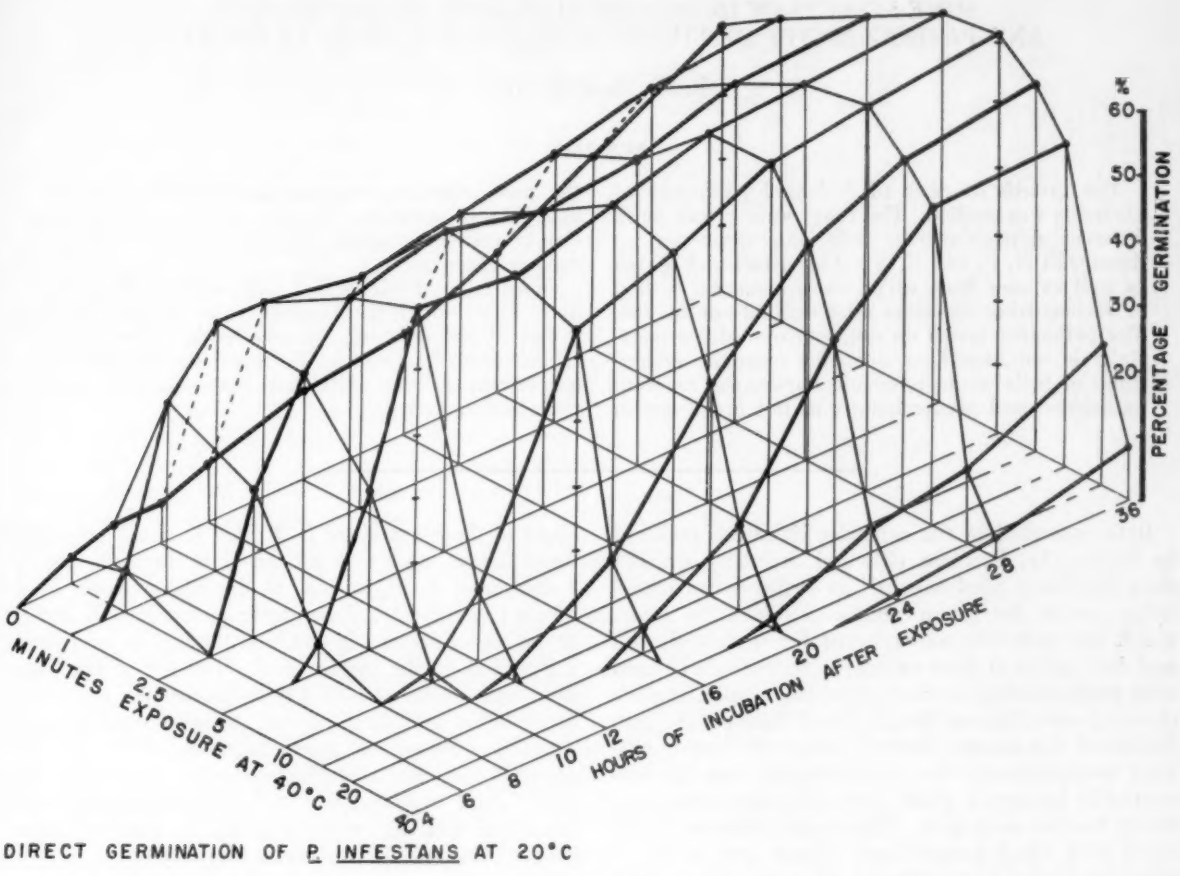



FIG. 2. Effect of the duration of exposure of sporangia at 40°C on the rate of direct germination to 36 hours.

in water followed a much different pattern than that of direct germination on agar at 20°. Here the control lot germinated at a rapid rate, 22 per cent in 1 hour, 70 per cent at 6 hours, and a final germination of 89 per cent at 24 hours (Table 2). After as little as 1 minute of exposure at 40°C, germination was delayed so that only 3 per cent of the sporangia had germinated at 4 hours and only 38 per cent at 24 hours. Germination was reduced and delayed even more by heat treatments of 2.5 and 5 minutes and was negligible after a 10-minute exposure. An interesting phenomenon observed was that direct germination occurred with some of the sporangia that had failed to germinate indirectly. This was especially pronounced with the 2.5- and 5-minute heat exposures. It is interesting to note that the combined indirect and direct germination resulted in a total germination near that of the direct germination on agar. A single lot of spores was used in this experiment. Samples for germination counts on both water and agar were taken simultaneously.

Preliminary data indicate that at 50° the survival time of the spores was only a few seconds; at 37.5° the critical sampling period extended over an inconveniently long period. For this reason, 40° was a convenient temperature for this experiment.

DISCUSSION.—Previously reported research on the effect of pregermination exposure to high temperature has dealt with spores of low germinability. In the present study, no dormancy seems to be involved inasmuch as the sporangia were obtained from young cultures, and the principal effect on direct germination was in the rate at which germination occurred, not in the total amount of germination after 48 hours. No explanation is suggested for the different response of the 2 types of germination to exposure to high temperatures. These studies serve to illustrate the fallacy of germination trials of a fixed duration where the test materials are of varied background.

CAMP DETRICK
FREDERICK, MARYLAND

# SOME EFFECTS OF INORGANIC NUTRIENTS ON THE GROWTH AND PATHOGENICITY OF FIVE FUNGAL PATHOGENS OF GLADIOLUS [1]

B. H. Marshall, Jr. [2]

SUMMARY

The growth *in vitro* of 5 fungal pathogens of gladiolus was studied. The fungi were grown in 18 different mineral-sucrose solutions, consisting of those with N, P, and K in a 2-level factorial design as well as ones from which these elements, S, Mg, or various trace elements were omitted one by one. The pathogens tested do not appear to differ materially in nutritional requirements from the various fungi of little or no economic importance as plant pathogens used predominantly as test organisms in the past. Certain responses to trace element deficiencies or excesses, however, warrant investigation in nutrient solutions of higher purity than those used in these tests.

Inoculation of foliage and corms of Picardy gladiolus with inoculum prepared from fungus mats grown at the different nutrient levels failed to establish any clear relation between the nutrient requirements of the individual test organisms and their pathogenicity.

Data presented in the extensive literature reviewed by Foster (1), Perlman (6), and Steinberg (9, 10) show that fungi need most of the elements considered indispensable for green plants. Filamentous fungi, which are especially well-adapted for studies of mineral deficiencies at fixed carbohydrate levels, have been used predominantly in this type of work since Raulin's classical researches on the growth of *Aspergillus niger*. Studies of this nature, however, have been largely confined to a relatively few test organisms, and the economically important plant pathogens have been neglected for the most part. The study reported herein deals with fungi parasitic on foliage and corms of gladiolus or on both. These pathogens cause multimillion dollar losses annually.

MATERIALS AND METHODS. — *Nutrition tests.* — The parasitic fungi used were, in the probable order of their importance to the country as a whole, *Fusarium oxysporum* f. *gladioli* (Massey) Snyd. & Hans., *Botrytis gladiolorum* Timmerm., *Stromatinia gladioli* (Drayt.) Whet., *Curvularia lunata* (Wakk.) Boed., and *Stemphylium* sp. These organisms were grown on 100 ml of a mineral-sucrose solution in 200-ml flasks for 4, 7, and 21 days. Stock solutions contained 2 levels each of N, P, and K; the low-level solution contained N at 14.0, P at 9.3, and K at 7.8 ppm and the high-level solution 10 times as much. Mg and S were included at concentrations of 24 and 32 ppm, respectively, and the concentrations of minor elements in ppm were Fe, 2.0; Zn, 1.8; Cu, 0.40; Mn, 0.20; and Mo, 0.18. The salts used as sources of macroelements were Ca $(NO_3)_2 \cdot 4H_2O$, $KH_2PO_4$, $NaH_2PO_4 \cdot H_2O$, KCl, $K_2SO_4$, $MgSO_4 \cdot 7H_2O$, $MgCl_2$, and $Na_2SO_4$. The trace elements were obtained from $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $MnCl_2$, and $H_2MoO_4$. A total of 18 different nutrient solutions was used. Eight of these contained N, P, and K in a 2-level factorial design, each with all the other elements listed; 7 contained N, P, and K at the higher level, each minus 1 of the other 7 elements; 1 contained N and P at the higher level; 1 N and K at the higher level; and 1 P and K at the higher level. The pH of the solutions was measured both before inoculation and after the different periods of growth. A buffer was not used. The salts were reagent grade chemicals. All water was distilled and only acid-cleansed pyrex glassware was used. Prior to inoculation and immediately following the addition of C.P. sucrose (100 g/l), all flasks were plugged and sterilized in an autoclave.

Botrytis, Stromatinia, or Stemphylium inoculum was prepared by growing the test fungus on potato-dextrose broth. After approximately 2 weeks' growth, the fungus mats were washed in sterile distilled water and ground in a sterilized Waring Blendor with just enough sterile distilled water to make a thick mycelial suspension. With a plantinum-wire loop, 1 loopful of this suspension was transferred to each flask. Fusarium or Curvularia inoculum consisted of a loopful of spore suspension made by washing an agar-slant culture with sterile distilled water. After inoculation, the flasks were stored on a laboratory table 4–21 days. At the end of the growth period, 50 ml of ethyl alcohol was added to each flask to terminate growth and to facilitate the filtering of all growth from the nutrient solutions. (The alcohol appears to coagulate the gelatinous matrix that forms in these solutions and thus considerably decreases the time required for filtration.) Filtration was accomplished with the aid of suction flasks, Buchner funnels, and preweighed filter paper discs. After filtering, the papers and fungus mats were dried for approximately 24 hours in an electric oven at 120°F and weighed.

Spore concentrations were determined by thoroughly agitating the flasks to suspend as many spores as possible and drawing off 10-ml samples with a pipette. Spore counts were made with the aid of a Levy corpuscle-counting chamber. Periods of growth were not

[1] Accepted for publication August 4, 1955.

[2] Formerly Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

uniform for the 5 test organisms. Therefore, each pathogen represents a separate study, and for the most part no correlations are attempted. Only the dry weight data were analyzed statistically.

*Pathogenicity tests.*—To determine the effect of nutrition on the pathogenicity of the fungi, foliage or corms of Picardy gladiolus were inoculated with mycelium-spore suspensions prepared from the fungus mats grown at the various nutrient levels under test. Cormel foliage was used as a substrate for the pathogens that primarily attack above-ground parts (Botrytis, Curvularia, and Stemphylium). Corm pieces were used in testing Fusarium and Stromatinia.

Cormels were sown thickly in 5-in. clay pots and grown for 7–8 weeks. The pots then were placed in an inoculation chamber 3 days prior to inoculation. The relative humidity of the chamber was maintained at approximately 95 per cent. The temperature was varied, depending upon the organism under test: 65° for Botrytis, 75° for Stemphylium, and 80°F for Curvularia. Inoculum was prepared from 18-day-old mats taken from 4 flasks containing each of the different nutrient solutions. The mats were ground in a Waring Blendor with 100 ml of distilled water. Each suspension was then sprayed on the foliage of plants in 5 pots with a common "Flit-gun." Plants in a total of 75 pots (5 pots for each of 15 different nutrient levels) were inoculated. Because of the limited capacity of the inoculation chamber and the minute amounts of growth in the solutions from which N, P, or Mg was omitted, fungi grown at these 3 nutrient levels were not included in this test. Pots remained in the inoculation chamber 5–10 days depending upon the time required for the foliage to show extensive infection. They were then returned to the greenhouse and observations were made the following day.

Fusarium and Stromatinia inocula were prepared as for the foliage inoculations, but less water was added so that the inoculum would be more concentrated. Corms were surface-sterilized with 1:1000 $HgCl_2$ for 5 minutes, rinsed in sterile water, air-dried, and quartered. The quarters were placed in sterilized moist chambers, inoculated by coating the freshly cut surface of each piece with inoculum, and incubated at laboratory temperature (75–80°F). Five quarters were inoculated with each of the 18 different inocula. Also included were a noninoculated check group and 5 quarters inoculated with a suspension prepared from mats grown on potato-dextrose broth. At 3-day intervals cross sections of the corm pieces were examined, and the extent of fungus penetration was measured with an ocular micrometer.

EXPERIMENTAL RESULTS.—*General results.*—Contrary to expectations, the nutrient solution containing all the macroelements and microelements each in the maximum concentration used in these experiments (hereafter referred to as the "complete" solution) did not necessarily produce the most vegetative growth (Fig. 1). Though they were usually superior to solutions having a lower concentration of N, P, or K, solutions

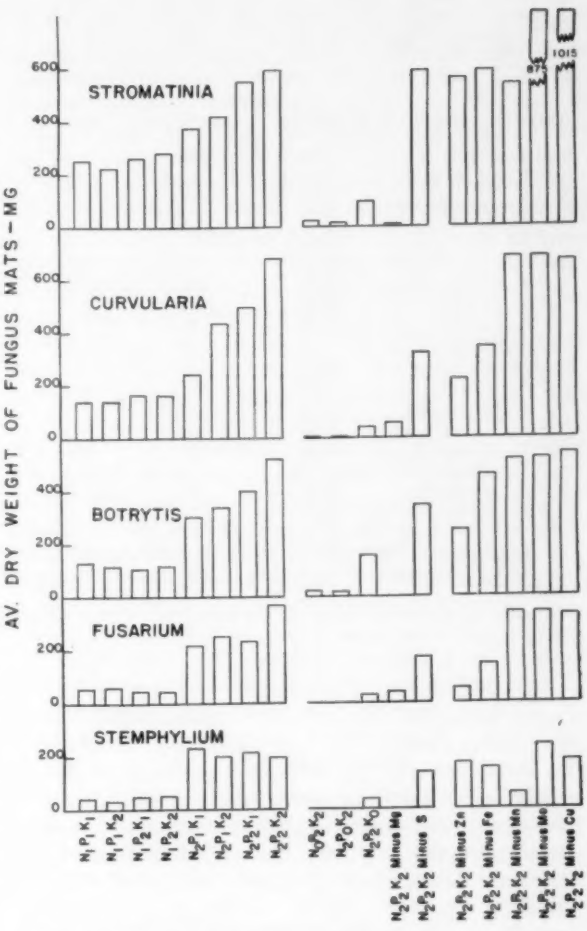



Fig. 1. Effect of inorganic nutrients on the growth of 5 fungal pathogens of gladiolus. All columns represent 14 days' growth except those for Stromatinia, which represent 21 days' growth.

from which Mo and Cu were omitted often produced as good or better growth. Sporulation, however, tended to be more prolific when the medium was "complete" (Table 1). Where N was at the low level, no significant increase in growth was obtained by raising P, K, or both to the high level. At the high level of N, both P and K had highly significant effects on growth. Raising the level of N significantly increased growth of Fusarium, Botrytis, and Curvularia, but not that of Stemphylium or Stromatinia. If Mg was omitted, little or no growth occurred. In the absence of Mg, there was even less growth of Botrytis and Stromatinia than where N or P was omitted. Curvularia and Fusarium required less Mg than did the other test organisms.

Except for Stromatinia, which seemed unaffected by the omission of Zn and Fe, and for Stemphylium, to which Mn seemed to be of particular importance, Zn and Fe, in that order, were the most growth-limiting of the minor elements. The omission of other microelements often resulted in reduced growth after 4 or

TABLE 1.—*Sporulation of 3 fungal pathogens of gladiolus grown 14 days in inorganic nutrient solutions*

| Nutrient solution | Spores per mm[a] of medium | | Color[a] rating of mats of |
|---|---|---|---|
| | Fusarium | Curvularia | Botrytis |
| $N_1P_1K_1$ | 4200 | 45 | 1 |
| $N_1P_1K_2$ | 400 | 25 | 1 |
| $N_1P_2K_1$ | 3600 | 90 | 1 |
| $N_1P_2K_2$ | 2500 | 45 | 1 |
| $N_2P_1K_1$ | 4900 | 90 | 3 |
| $N_2P_1K_2$ | 6750 | 70 | 5 |
| $N_2P_2K_1$ | 2100 | 95 | 5 |
| $N_2P_2K_2$ | 6900[b] | 230 | 5 |
| $N_0P_2K_2$ | 0 | 0 | 0 |
| $N_2P_0K_2$ | 150 | 0 | 0 |
| $N_2P_2K_0$ | 450 | 0 | 1 |
| $N_2P_2K_2$ minus S | 4500[b] | 50 | 4 |
| $N_2P_2K_2$ minus Mg | 350 | 0 | 0 |
| $N_2P_2K_2$ minus Cu | 4100[b] | 45 | 4 |
| $N_2P_2K_2$ minus Zn | 2800[b] | 40 | 2 |
| $N_2P_2K_2$ minus Fe | 4450[b] | 90 | 3 |
| $N_2P_2K_2$ minus Mn | 4700[b] | 0 | 5 |
| $N_2P_2K_2$ minus Mo | 6400[b] | 75 | 5 |

[a] Rated on the shade of gray or black of the fungus mats; the darker the color (5 is darkest), the greater the sporulation.

[b] Includes a few macrospores not found in other solutions.

7 days' incubation. If the growth period was extended to 14 days, however, these omissions tended to have no effect. Absence of Cu and Mo usually produced no significant changes while the omission of Mn resulted in a significant decrease in growth of Stemphylium and Stromatinia. These 2 fungi, on the other hand, were the only ones that failed to produce significantly less growth on media from which S was omitted.

The dry weights of fungus mats grown in the absence of the various elements (Fig. 1) indicate that N, P, Mg, and K, in that order, were the most growth-limiting. Growth and pH changes in the substrate were closely correlated. Where the change in pH was relatively large, growth was heavy, and *vice versa*. The average initial pH of the medium was 3.9; usually the medium became more alkaline as growth increased. In the early stages of growth, Fusarium cultures became slightly more acid, but if growth continued for 2 weeks or more, the medium invariably shifted toward alkalinity. In Botrytis cultures, however, the medium became more acid in the early stages of growth, and with a longer growth period the acidity continued to increase.

*Effects of nutrients on individual organisms.*—1) *Fusarium.*—In the absence of Mg, Fusarium made a surprising amount of growth in 14 days. At the low level of N, sporulation was greater if P and K were also low. If either was raised to the high level, sporulation decreased. The low level of P seemed sufficient for maximum sporulation, but higher concentrations of N and K were required. Except in solutions to which no S was added, macrospores were not formed unless macronutrients were complete. Spore production was lowered in the absence of any 1 of the minor elements.

The effects on pigmentation of the "complete" solutions and of the solutions from which Mo and Mn were omitted were striking. A combination of purple and orange and purple and red pigments, respectively, developed in these mats. There were also slight tinges of purple, orange, or both in the mycelial mats growing in solutions in which P and K were low. Where N was low, the thin surface growth was dark purple. The medium itself remained colorless.

2) *Botrytis.*—The only organism in the test that had increased the acidity of the medium after 14 days' growth was Botrytis. There was little or no increase in growth after 7 days if N was low. Where N was high, there was twice as much growth in 14 days as in 7. Low P and/or K had little effect on conidial production if N was high. Conversely, where N was low, conidial production was not increased by raising the levels of P and/or K. There was considerable growth in the absence of K, but the number of conidia was very small. Zn was the only trace element whose absence significantly decreased growth. The main effect of Fe omission was a decrease in sporulation. There was practically no growth in the absence of Mg.

3) *Stromatinia.*—There was a direct correlation between growth of Stromatinia and pH. As growth increased, pH increased from approximately 3.9 to 6.1. Zn and Fe requirements were apparently slight. Though a reduced growth rate was observed in Fe-deficient solutions after 4 days, it was not evident after an added growth period of 17 days. The solution from which Cu was omitted was consistently the best medium for growth, suggesting that Stromatinia is particularly sensitive to Cu and that the 0.4 ppm used, together with that present as an impurity, may have been excessive. Fungus mats were largely gray-black, dotted with very small black sclerotia. The solutions remained colorless. Where N, P, or K were omitted, growth was subsurface. In the solution without Mg there was practically no growth. Media containing N at the low level produced thin, shiny, black mycelial mats having an iridescent appearance.

4) *Curvularia.*—The reaction of Curvularia to N, P, and K was similar to that of the other test organisms. There were no significant differences between vegetative growth in the "complete" solution and in solutions from which Cu, Mn, or Mo was omitted; however, Mn does appear to be necessary for sporulation. In fact, sporulation was greatly decreased in any nutrient solution containing macroelements or microelements in a concentration less than that of the "complete" solution. The omission of Fe and Zn reduced growth ½ and ⅔, respectively. Curvularia showed less requirement for Mg than any other fungus in these tests. All fungus mats were shades of gray-white or black. The medium remained colorless. The pH was highest (7.5) in solutions in which Curvularia was grown.

5) *Stemphylium.*—Varying the level of P and K at a constant N level produced no appreciable changes in growth of Stemphylium. Significant differences were obtained only between the high and the low levels of

N. At the high level of N, growth was better than that in the "complete" solution if P or K, or both were low. Though the differences were not significant, this pattern was considered unusual enough and consistent enough to warrant mention. A striking requirement for Mn was shown during the 14-day growth period; otherwise the minor elements were seemingly of little importance. Solutions from which Cu was omitted had produced twice as much growth as the "complete" solution by the end of the 7-day growth period. No superiority was evident, however, if the cultures were grown for 7 additional days. No sporulation occurred at any nutrient level. Cultures were predominantly black.

*Pathogenicity tests.*—Nothing requiring special comment was found in the pathogenicity tests. The results were erratic, and except for an effect of N concentration on the severity of Botrytis infection showed no clear influence of nutrition of the pathogen on its pathogenicity.

Discussion.—The technique used in these tests was not sufficiently refined to give an accurate picture of the essentiality of the minor elements. Foster (1) states that Zn- and Fe-deficient cultures should not yield a dry weight of more than 10–15 mg per 50 ml of solution (because of unavoidable impurities), whereas Cu-, Mn-, and Mo-deficient cultures might yield considerably more. As can be seen in Figure 1, growth obtained from these cultures in this experiment was far greater. This is undoubtedly due to the minute quantities of these trace elements occurring as impurities in the distilled water and chemicals. Without the use of special techniques, considered impractical in this particular experiment, we could not expect to get the extremely small yields mentioned by Foster. Yields significantly less than those in the "complete" solution usually can be obtained, however, if Zn, Fe, and occasionally other trace elements, particularly Mn, are omitted; but the response depends upon the organism involved. In general, Zn and Fe are the trace elements required in largest amounts by fungi, and consequently it is easier to demonstrate their deficiencies. According to Foster (1), Zn enables the organism to utilize its energy source more efficiently. For the most part, however, our knowledge of the heavy-metal nutrition of fungi is largely confined to the amount of growth, physical appearance, pigmentation, etc. The mechanism of the action of metals is practically unknown.

In the case of macroelements such as K, impurities alone are insufficient to account for the relatively high yields obtained in their absence. It is known that Rb and possibly Cs may take the place of K for Hyphomycetes (Nageli cited by Rahn (7)) and this might help to explain the decidedly better growth where K was omitted than where N or P was missing. The presence of these alkali metals as contaminants is possible, but it seems certain that the fungi studied have a lower requirement for K than for N, P, or Mg.

The acid-alkaline reversal of pH reported by Golding (2) was observed to some extent in these tests, but its occurrence was not uniform for all of the fungi tested. Although all organisms except Botrytis had decreased the acidity of the solutions after 2 weeks' growth, reversal from a pH lower than that noted prior to inoculation could not be detected after 7 days for any fungus but Fusarium. It is entirely possible, however, that reversal took place several days earlier. Golding states that the growth of Penicillium causes an acid reaction until all of the carbohydrate is used up, and then the reaction reverses toward the alkaline side. Thus it might be reasoned that Botrytis either has a smaller carbohydrate requirement than the other test organisms or produces an acid substance that prevents any alkaline reversal. At any rate, the importance of acidity is open to question and apparently varies with the organism under test. Perlman (5), working with *Sclerotium delphinii*, found that the metabolism of available carbohydrate was greatly reduced when the pH of the medium fell below 2.3. If the pH of the medium was kept above 4.0, the carbohydrate was metabolized faster and also oxalic acid production was increased. On the other hand, Steinberg (8), using *Aspergillus niger* as the test organism, states that acidity is of minor importance and is effective chiefly through helping to minimize trace-element deficiencies.

In the pathogenicity tests, the rate of penetration of the corm by Fusarium was considerably greater than that by Stromatinia. It must be pointed out, however, that after 6 days, Stromatinia penetration had almost ceased. This resulted from the formation of a wound periderm that first slowed and then stopped penetration. Fusarium was not "walled-off" in a like manner because its more rapid growth rate prevented the formation of this protective layer of cells. This occurrence has been reported in detail elsewhere (4). It is believed possible to prevent the formation of a restrictive periderm in Stromatinia tests by lowering the test temperatures to perhaps 60 or 65°F. Such a temperature should be less favorable to periderm formation than to growth of this particular organism. The pathogenicity tests are, for the most part, inconclusive. It would appear, however, that nutrients available to a fungus in the past have little to do with its present pathogenicity. These results are supported somewhat by the work of Leben and Keitt (3) on the nutrition of *Venturia inaequalis*. After studying 4 pathogenic and 3 nonpathogenic strains, they reached the conclusion that no differences in C, N, or vitamin nutrition of the various strains were clearly associated with the pathogenic capabilities of the organisms.

U. S. Plant Industry Station
Beltsville, Maryland

LITERATURE CITED

1. Foster, J. W. 1939. The heavy metal nutrition of fungi. Bot. Rev. **5**: 207-239.
2. Golding, N. W. 1934. The growth of Penicillium roqueforti on synthetic media. Jour. Dairy Sci. **17**:

61-74.
3. LEBEN, C., AND G. W. KEITT. 1948. Venturia inaequalis (Cke.) Wint. V. The influence of carbon and nitrogen sources and vitamins on growth in vitro. Amer. Jour. Bot. **35**: 337-343.
4. MARSHALL, B. H., JR. 1953. Relation of wound periderm in gladiolus corms to penetration by fusarium oxysporum f. gladioli. Phytopathology **43**: 425-431.
5. PERLMAN, D. 1948. On the nutrition of Sclerotium delphinii. Amer. Jour. Bot. **35**: 360-363.
6. PERLMAN, D. 1949. Effects of minor elements on the physiology of fungi. Bot. Rev. **15**: 195-220.
7. RAHN, O. 1936. Substitutes for potassium in metabolism of the lowest fungi. Jour. Bact. **32**: 393-399.
8. STEINBERG, R. A. 1937. The role of molybdenum in the utilization of ammonium and nitrate nitrogen by Aspergillus niger. Jour. Agr. Res. **55**: 891-902.
9. STEINBERG, R. A. 1939. Growth of fungi in synthetic nutrient solutions. Bot. Rev. **5**: 327-350.
10. STEINBERG, R. A. 1950 . Growth of fungi in synthetic nutrient solutions. II. Bot. Rev. **16**: 208-228.

# HOST-PARASITE INTERACTION IN FLAX RUST—ITS GENETICS AND OTHER IMPLICATIONS [1]

H. H. Flor [2]

SUMMARY

The pathogenicity of 67 $F_2$ cultures of a cross of race 6 with race 22 of the flax rust pathogen was determined on 32 varieties of flax that had been selected as carrying single genes for rust reaction. Two varieties were resistant and 6 were susceptible to all $F_2$ cultures. On 23 of the 24 varieties on which the $F_2$ cultures segregated for pathogenicity, the ratio of avirulent to virulent cultures approximated the 3:1 expected if virulence on each was conditioned by a pair of recessive genes. On Ottawa 770B, 2 pairs of genes may have conditioned pathogenicity. Fifty-four pathogenic races were identified from the 67 cultures.

Host–parasite interaction in flax rust may be explained by assuming a gene-for-gene relationship between rust reaction in the host and pathogenicity in the parasite. Pustule type, the criterion of both reaction and pathogenicity, is conditioned by specific pairs of genes, one in the host and the other in the parasite. In flax and the flax rust fungus, 25 such pairs of genes have been identified.

Because of the gene-for-gene relationship between reaction in the host and pathogenicity in the parasite, the recessive gene complement of a uredial clone (culture) is established by determining its pathogenicity on differential varieties with single rust-conditioning genes. The homozygosity or heterozygosity of the dominant genes is established by selfing the uredial clone. Thus, a method for identifying the pathogenic genotype of races of the rust fungi has been devised. This makes possible the use of the biotype as the basic concept of race.

The gene-for-gene relationship of rust reaction and pathogenicity in host and parasite facilitates the development of rust-resistant varieties and opens up new approaches to studies of the origin of new races, mutation for rust reaction in the host and pathogenicity in the parasite, the evaluation of epidemiology data, and the nature of resistance.

Notwithstanding extensive investigations on physiologic specialization, pathogenic variability, the nature of resistance, and epidemiology of the cereal rusts, newly developed rust-resistant varieties of wheat, oats, and flax often succumb to rust after a few years of commercial production. This suggests the need for a new approach to studies on the rusts.

In 1942, Flor (4) pointed out the gene-for-gene relationship between pathogenicity in the flax rust fungus, *Melampsora lini* (Pers.) Lév. and rust reaction in the host, *Linum usitatissimum* L. Later studies not only verified this finding, but also showed the relationship to be highly specific.

$F_2$ cultures of race 6 × race 24 segregated for pathogenicity on the varieties Buda, Akmolinsk, and Bombay (3). Virulence was recessive. On Akmolinsk and Bombay, varieties having 1 gene for rust reaction, pathogenicity was conditioned by single genes. On Buda, which has 2 genes for rust reaction, pathogenicity was conditioned by 2 genes. The gene for pathogenicity on Akmolinsk appeared to be linked with 1 of the genes for pathogenicity on Buda.

The parent races in the cross of race 22 with race 24 attacked 15 of 16 differential varieties (4). $F_1$ cultures attacked only the 3 differentials susceptible to both parent races. From 133 $F_2$ cultures, 64 pathogenically different races were identified. Virulence was recessive and conditioned by 1 gene on Abyssinian, Akmolinsk, Bombay, Kenya, Leona, Newland, Ottawa 770B, Pale Blue Crimped, and Tammes Pale Blue, varieties having 1 gene for resistance to the avirulent parent race. Two genes conditioned virulence on Bolley Golden and Italia Roma, varieties having 2 genes for resistance to race 24, and 3 genes conditioned virulence on Morye, a variety having 3 genes

[1] Accepted for publication August 3, 1955.
Cooperative investigations of Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station. Published with the approval of the Director of the North Dakota Agricultural Experiment Station.
[2] Pathologist, Field Crops Research Branch, Fargo, North Dakota.

for resistance to race 24. The genes for virulence on Abyssinian, Akmolinsk, and Leona, and on Kenya and Pale Blue Crimped were so closely linked as to be inherited as units. These studies indicated that 1 of the genes for virulence on Bolley Golden was identical or closely linked with 1 of the genes for virulence on Italia Roma. Likewise, the genes for virulence on Tammes Pale Blue either were identical or closely linked with 1 of the 3 pairs of genes conditioning virulence on Morye.

Although the pathogenicity of 98 $F_2$ cultures secured from 3 populations of the cross of race 6 with race 22 was determined (4), that number of cultures was considered too small for statistical analysis of the interaction of the genes for pathogenicity. Differences in the segregating ratios between the race 22 $\times$ race 24 cultures and the race 6 $\times$ race 22 cultures on Tammes Pale Blue and Morye, however, emphasized the gene-for-gene relationship between reaction in the host and pathogenicity in the parasite. In the cross of race 22 $\times$ race 24, pathogenicity on Tammes Pale Blue and Morye was conditioned by 1 and 3 genes, respectively. In the cross of race 6 $\times$ race 22, pathogenicity on Tammes Pale Blue was conditioned by 2 genes and pathogenicity on Morye by 4 genes. The Koto gene had not been discovered, but subsequently (6) it was determined that both Tammes Pale Blue and Morye carry the Koto gene. Koto is susceptible to races 22 and 24 and resistant to race 6. Consequently, the Koto gene in Tammes Pale Blue and Morye was not effective against the race 22 $\times$ race 24 hybrids but was effective against the race 6 $\times$ race 22 hybrids.

MATERIALS AND METHODS.—Studies on the genetics of pathogenicity in the rusts are laborious and exacting. Statistically significant data on the interaction of genes for virulence can be secured from fewer cultures if the differential varieties possess single rust-conditioning genes than if they possess 2, 3, 4, or more. Consequently, a new series of flax rust differentials was developed (6). Each new differential apparently possesses a single gene for reaction to North American races. The new differentials were selected on the basis of the possession of genes that 1) condition resistance to North American races, 2) condition reaction in commercial varieties, and 3) identify races on a world-wide basis.

Telia developed from the $F_1$ culture of race 6 $\times$ race 22, Hybrid A, were found to be viable after 7 years of storage at about 3°C, and 67 $F_2$ cultures were secured. In pathogenicity studies on the rusts, the interaction is between the dikaryotic (2n) urediospore culture and the diploid (2n) host. Because the urediospore is the repeating and pathogenic phase of the flax rust fungus, it has an advantage in genetic studies over the smuts and higher plants in that each $F_2$ variant may be maintained indefinitely as a uredial clone by periodic passage through the host. As the new lines of flax possessing single rust-conditioning genes were developed, their reaction to the 67 $F_2$ cultures was determined. These results were verified in a final test of the reaction of 32 varieties to each culture.[3] Although some of these varieties do not serve to identify numbered races, all that differentiate the $F_2$ cultures are of equal importance in a study on the genetics of pathogenicity.

RESULTS.—*Pathogenicity studies.*—The reaction of 32 varieties of flax to 67 $F_2$ cultures was determined. Two varieties, Bombay and J.W.S., were resistant to all cultures; and 6 varieties, Akmolinsk, Barnes, Bison, Victory A, Wilden, and Williston Brown, were susceptible to all cultures. The 24 varieties on which the cultures segregated for pathogenicity fell into 12 differential groups (Table 1). Based on a dichotomous classification, 54 pathogenic races were identified among the 67 $F_2$ cultures. Theoretically, $2^{12}$ or 4096 races were obtainable from this cross if enough cultures had been studied.

On all the differential varieties except Ottawa 770B, the cultures segregated into ratios satisfactorily approximating the 3 avirulent to 1 virulent expected if virulence on each was conditioned by a single pair of recessive genes. This is further confirmation of the possession by each of these varieties of a single gene for resistance to North American race 6.

The occurrence of 7 virulent to 60 avirulent cultures on Ottawa 770B is difficult to explain on a simple Mendelian basis. In tests with the cross of race 22 $\times$ race 24, 33 of 133 $F_2$ hybrid cultures attacked Ottawa 770B (4), almost a perfect 1:3 ratio. In the previous tests of race 6 $\times$ race 22 (4), 10 of 74 $F_2$ cultures of Hybrid A, 1 of 16 $F_2$ cultures of Hybrid B, and 2 of 8 $F_2$ cultures of Hybrid C attacked Ottawa 770B. Henry (7), Myers (10), and Flor (5) found that in Ottawa 770B, 1 gene conditioned resistance to North American races. Kerr (9) found, however, that in addition to the *L* gene which conditions resistance to North American races, Ottawa 770B possesses a gene in the *N* series of allelomorphs conditioning resistance to some Australian races. Although the gene for avirulence on varieties possessing the *N* gene of Ottawa 770B is not known to occur in North American races, it is possible that race 6 was heterozygous for it.

The $\chi^2$ values were calculated for association of pathogenic characters on the 12 varietal groups. These data indicated that pathogenicity on 9 varieties or groups of varieties was inherited independently or was too loosely linked to be evident in the analysis of the limited number of cultures studied. Pathogenicity was inherited independently on Ottawa 770B, Dakota, Birio, and Burke and was inherited as an independent unit on Abyssinian, Koto, Leona, Ward, and Wells; on Bowman, Clay, Grant, and Minnesota selection; and on Argentine selection, Cortland, and Lino 6899 M.A. Pathogenicity on Polk and Marshall was closely linked, and pathogenicity on these varieties was loosely linked with that on Cass. Pathogenicity was inherited as a unit on B. Golden selection, Kenya,

[3] Six cultures were lost before Towner (C. I. 1561) was isolated.

TABLE 1.—*Reaction of varieties and lines of flax to 67 cultures of a cross of race 6 with race 22 of Melampsora lini*

| No. cultures with indicated pathogenicity | Reaction[a] on indicated differential-variety group[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | S | S | R | R | S | R | R | R | S | S | R | S |
| 1 | S | R | S | R | S | R | R | R | R | S | R | S |
| 1 | S | R | R | S | R | R | R | R | S | S | R | S |
| 1 | S | R | R | R | S | S | S | R | S | S | R | R |
| 1 | S | R | R | R | S | R | R | R | R | R | R | R |
| 1 | S | R | R | R | R | R | R | R | S | S | R | R |
| 1 | S | R | R | R | R | R | R | R | R | R | R | R |
| 3 | R | S | S | R | R | S | S | R | R | R | R | R |
| 1 | R | S | S | R | R | R | R | S | S | S | R | R |
| 1 | R | S | S | R | R | R | R | R | R | R | S | R |
| 1 | R | S | R | S | R | R | R | S | R | R | R | S |
| 1 | R | S | R | R | S | R | R | S | S | R | R | R |
| 1 | R | S | R | R | S | R | R | R | R | R | R | R |
| 1 | R | S | R | R | R | S | S | R | S | | S | R |
| 1 | R | S | R | R | R | S | S | R | R | R | R | R |
| 1 | R | S | R | R | R | R | S | S | R | S | R | R |
| 1 | R | S | R | R | R | R | R | S | R | S | R | R |
| 1 | R | S | R | R | R | R | R | R | S | S | R | R |
| 1 | R | S | R | R | R | R | R | R | R | R | R | R |
| 1 | R | R | S | S | S | R | R | S | S | R | S | R |
| 1 | R | R | S | S | R | S | S | R | R | S | S | S |
| 1 | R | R | S | S | R | S | S | R | R | R | R | S |
| 1 | R | R | S | S | R | R | R | R | S | S | R | R |
| 1 | R | R | S | R | S | S | S | S | R | R | R | R |
| 2 | R | R | S | R | S | S | S | R | R | R | R | R |
| 1 | R | R | S | R | S | R | R | R | R | | R | R |
| 1 | R | R | S | R | R | S | S | R | S | R | R | S |
| 1 | R | R | S | R | R | S | S | R | R | R | R | S |
| 1 | R | R | S | R | R | R | R | R | S | S | S | R |
| 1 | R | R | S | R | R | R | R | R | S | S | S | S |
| 1 | R | R | S | R | R | R | R | R | R | R | S | S |
| 1 | R | R | S | R | R | R | R | R | R | | S | R |
| 1 | R | R | S | R | R | R | R | R | R | R | R | S |
| 1 | R | R | R | S | S | S | S | R | S | S | R | R |
| 1 | R | R | R | S | S | R | R | R | R | R | R | S |
| 1 | R | R | R | S | R | S | S | S | S | R | R | S |
| 1 | R | R | R | S | R | S | S | R | R | R | R | S |
| 1 | R | R | R | S | R | S | S | R | R | R | R | R |
| 1 | R | R | R | S | R | R | R | S | R | S | R | S |
| 1 | R | R | R | S | R | R | R | R | R | R | S | R |
| 1 | R | R | R | S | R | R | R | R | R | | R | R |
| 1 | R | R | R | R | S | R | R | S | S | R | R | S |
| 1 | R | R | R | R | S | R | R | S | R | | S | S |
| 1 | R | R | R | R | S | R | R | R | S | S | S | R |
| 1 | R | R | R | R | S | R | R | R | R | S | R | R |
| 1 | R | R | R | R | R | R | R | S | S | R | R | S |
| 2 | R | R | R | R | R | R | R | S | R | R | R | R |
| 1 | R | R | R | R | R | R | R | S | S | | R | S |
| 1 | R | R | R | R | R | R | R | R | R | R | S | S |
| 2 | R | R | R | R | R | R | R | R | R | R | S | R |
| 2 | R | R | R | R | R | R | R | R | R | R | R | S |
| 8 | R | R | R | R | R | R | R | R | R | R | R | R |
| No. virulent cultures | 7 | 15 | 21 | 14 | 17 | 17 | 19 | 17 | 19 | 16 | 15 | 22 |
| $\chi^2$ (3:1)[c] | 7.56 | .24 | 1.43 | .60 | .01 | .01 | .40 | .01 | .40 | .04 | .24 | 2.19 |
| $\chi^2$ (15:1)[c] | 2.02 | | | | | | | | | | | |

[a] S = susceptible; R = resistant.

[b] 1 = Ottawa 770B; 2 = Dakota; 3 = Cass; 4 = Abyssinian, Koto, Leona, Ward, and Wells; 5 = Bowman, Clay, Grant, and Minnesota sel.; 6 = Polk; 7 = Marshall; 8 = Birio; 9 = B. Golden sel., Kenya, Norman, and Pale Blue Crimped; 10 = Towner; 11 = Argentine sel., Cortland, and Lino 6899 M.A.; and 12 = Burke. All varieties listed were resistant to race 6 and susceptible to race 22.

[c] Ratio refers to ratio of avirulent to virulent cultures. $\chi^2$ at 5 per cent point, 3.84; at 1 per cent point, 6.64.

Norman, and Pale Blue Crimped, and pathogenicity on this varietal group appeared to be loosely linked with that on Towner.

No crossing over was observed between the genes for virulence on Abyssinian, Koto, Leona, Ward, and Wells; on Bowman, Clay, Grant, and Minnesota selection; on B. Golden selection, Kenya, Norman, and Pale Blue Crimped; and on Argentine selection, Cortland, and Lino 6899 M.A. Hence the assumption that a single gene conditions pathogenicity on all the varieties in each of these 4 group satisfactorily explains the results. In the study of race 22 × race 24 (4), however, pathogenicity on Akmolinsk, susceptible to all cultures of race 6 × race 22 (Hybrid A), was inherited as a unit with pathogenicity on Abyssinian and Leona. Since Abyssinian, Akmolinsk, Koto, Leona, and Ward are differentiated from each other by North American races, they possess different rust-conditioning genes. Consequently, the assumption that the genes for pathogenicity on these varieties are so closely linked that they are inherited as a unit appears to be the more simple explanation. Wells, derived from Pale Verbena (C. I. 416-3), has had the reaction of Leona to all races. These 2 varieties may have an identical gene for rust reaction.

Bowman, Clay, Grant, and Minnesota selection are differentiated by North American races. All of these varieties have not been studied genetically, however, and there may be some duplication of genes. For example, Clay is susceptible and Bowman is resistant to race 7, but these 2 varieties react alike to many races. The possibility that the resistance of Bowman to race 7 is due to a second gene effective against race 7 but not effective against most other North American races has not been determined.

Myers (10) reported 2 genes conditioning rust reaction in Minnesota selection, C. I. 438. One gene conditioned immunity and was allelic to the *L* gene in Ottawa 770B. The second gene conditioned resistance and was allelic to the *M* gene in Newland. Barnes (C. I. 1190), a variety carrying an *L* gene, has been isolated from Minnesota selection × Bison. As Barnes was susceptible to parent races 6 and 22, the resistance of Minnesota selection to certain $F_2$ cultures of this cross was conditioned by the *M* gene only. Consequently, the $F_2$ cultures did not differentiate lines carrying both the *L* and the *M* genes of Minnesota selection from lines carrying only the *M* gene.

Bolley Golden selection, Kenya, Norman, and Pale Blue Crimped are differentiated by North American races. The uniqueness of the genes in Bolley Golden selection and Norman has not been established.

Argentine selection, Cortland, and Lino 6899 M.A. have reacted alike to all North American and hybrid races. Although Straib (12) separated European races by the differential reactions of Argentine selection and Lino 6899 M.A., these varieties may possess the same major rust-conditioning gene. Cortland possesses 1 of the genes conditioning rust reaction in Pale Verbena.

*Genetics of host-parasite interaction.*—The genes for rust reaction in the host are identified by the pathogenicity of specific races of the parasite. And conversely, the genes for pathogenicity in the parasite are identified by the reaction of specific varieties of the host. Varieties having the same reaction to all races are considered to possess identical genes for rust reaction. Races having the same pathogenicity on a variety are considered to possess identical genes for pathogenicity on that variety. The identity of genes for rust reaction in different varieties is provisional only. In these studies, most of the genes for virulence on the differential varieties were derived from race 22. If the genes for resistance to North American races in 2 varieties were allelic and the genes for virulence on these varieties were closely linked in race 22, the varieties would be considered to have identical genes for rust reaction.

Genes for rust reaction in flax have been designated by the symbols *L*, *M*, and *N*, and members of allelic series by superscripts (5, 10). All genes designated by the symbols *L* or *M* have been allelic, but crossing over between some of the genes designated by the symbol *N* has indicated the occurrence of 2 series of linked alleles in the chromosome carrying the *N* genes (5). The relation of some of the *N* genes has been determined. The symbol *N* has been retained for those allelic to the rust-conditioning gene in Bombay. The symbol *P* has been assigned to the genes lying at the other locus. Rust reaction in Clay is conditioned by an independently inherited gene to which the symbol *K* has been assigned. The relation and uniqueness of some genes have not yet been established.

Formerly, the symbols *A* and *V* were used to designate genes for avirulence and virulence, respectively, in the rust fungus, with subscripts indicating the differential variety on which the gene conditioned pathogenicity (4). A new system of gene designation now has been devised to show the specificity of interaction of the genes in host and parasite. The symbol of the gene in flax with which the gene in the rust fungus interacts is used as the subscript to *A* and *V* to indicate this specific relationship. In Table 2 is given the genotype, if established, of the new flax rust differentials, each possessing a single rust-conditioning gene, and the old and new genotype suggested for race 6.

Stakman et al (11) noted the desirability of using the biotype as the basis for concepts regarding races. The gene-for-gene relationship between reaction in the host and pathogenicity in the parasite makes it possible to approach more closely the biotype as the basis for race. A routine test on differentials with single

TABLE 2.—*Probable flax and rust genotypes for a new set of differential varieties in relation to race 6*

| Differential variety | C. I. number | Flax genotype[a] | | | | Rust genotype race 6 | |
|---|---|---|---|---|---|---|---|
| | | | | | | New | Old |
| Ottawa 770B | 355 | kk | LL | mm | np/np[b] | $A_L$ $A_L$ | $A_o$ $A_o$ |
| Dakota | 1071 | kk | ll | MM | np/np | $A_M$ $A_M$ | $A_n$ $A_n$ |
| Bombay | 42 | kk | ll | mm | Np/Np | $A_N$ $A_N$ | $A_{bom}$ $A_{bom}$ |
| Stewart | 1072 | kk | $L^2L^2$ | mm | np/np | $A_L{}^2$ $A_L{}^2$ | $A_{Iws}$ $A_{Iws}$ |
| Cass | 1182 | kk | ll | $M^3M^3$ | np/np | $A_M{}^3$ $A_M{}^3$ | $A_{bg}$ $A_{bg}$ |
| Koto | 842 | kk | ll | mm | nP/nP | $A_P$ $A_P$ | $A_{tpb}{}^1$ $A_{tpb}{}^1$ |
| Clay | 1188 | KK | ll | mm | np/np | $A_K$ $A_K$ | $A_{ar}{}^1$ $A_{ar}{}^1$ |
| Polk | 1191 | kk | ll | mm | $N^1p/N^1p$ | $A_N{}^1$ $A_N{}^1$ | $A_{tpb}$ $A_{tpb}$ |
| Birio | 1085 | kk | $L^6L^6$ | mm | np/np | $A_L{}^6$ $A_L{}^6$ | $A_{ar}$ $A_{ar}$ |
| Kenya | 709 | kk | $L^4L^4$ | mm | np/np | $A_L{}^4$ $A_L{}^4$ | $A_k$ $A_k$ |
| Akmolinsk | 515 | kk | ll | mm | $nP^1/nP^1$ | $A_P{}^1$ $a_P{}^1$ | $A_{ak}$ $A_{ak}$ |
| Abyssinian | 701 | kk | ll | mm | $nP^2/nP^2$ | $A_P{}^2$ $A_P{}^2$ | $A_{ab}$ $A_{ab}$ |
| Leona | 836 | kk | ll | mm | $nP^3/nP^3$ | $A_P{}^3$ $A_P{}^3$ | $A_{1e}$ $A_{1e}$ |
| Wilden | 1193 | kk | $L^5L^5$ | mm | np/np | $a_L{}^5$ $a_L{}^5$ | |
| Williston Brown | 803 | kk | ll | $M^1M^1$ | np/np | $V_M{}^1$ $V_M{}^1$ | |
| Victory A | 1170 | kk | ll | $M^4M^4$ | np/np | $A_M{}^4$ $a_M{}^4$ | |
| Bowman | 1184 | | | | [c] | A A | |
| Bison | 389 | kk | $L^9L^9$ | mm | np/np | $a_L{}^9$ $a_L{}^9$ | |
| Burke | 1180 | kk | $L^1L^1$ | mm | np/np | $A_L{}^1$ $A_L{}^1$ | $A_{bu}$ $A_{bu}$ |
| Ward | 1181 | kk | ll | $M^2M^2$ | np/np | $A_M{}^2$ $A_M{}^2$ | $A_{bu}{}^1$ $A_{bu}{}^1$ |
| Pale Blue Crimped | 647 | kk | $L^8L^8$ | mm | np/np | $A_L{}^8$ $A_L{}^8$ | $A_P$ $A_P$ |
| Norman | 1186 | | | | [c] | A A | $A_{1r}$ $A_{1r}$ |
| Bolley Golden sel. | 1183 | kk | $L^{10}L^{10}$ | mm | np/np | $A_{L10}$ $A_{L10}$ | |
| Grant | 1194 | | | | [c] | A A | $A_{tpb}$ $A_{tpb}$ |
| Marshall | 1335 | kk | ll | mm | $N^2p/N^2p$ | $A_N{}^2$ $A_N{}^2$ | |
| Argentine sel. | 705-1 | | | | [c] | A A | |
| Lino M.A. 6899 | 868 | | | | [c] | A A | |
| Cortland | 1512 | kk | ll | $M^5M^5$ | np/np | $A_M{}^5$ $A_M{}^5$ | $A_{1e}$ $A_{1e}$ |
| Wells | 1513 | kk | ll | mm | $nP^3/nP^3$ | $A_P{}^3$ $A_P{}^3$ | |
| Minnesota sel. | 438 | | $L^7L^7$ | | [c] | A A | |
| Barnes | 1190 | kk | $L^7L^7$ | mm | np/np | $a_L{}^7$ $a_L{}^7$ | |
| Towner | 1561 | kk | $L^8L^8$ | mm | np/np | $A_L{}^8$ $A_L{}^8$ | |

[a] Most of the new differential varieties were derived from hybrids of Bison with the old differentials. As Bison has been susceptible to all North American races, the presence or absence of the Bison gene $L^9$ in the new differentials with *K*, *M*, *N*, *P*, and not placed rust-conditioning genes has not been determined.

[b] Kerr (9) reports an additional gene in the N chromosome conditioning resistance to certain Australian races.

[c] Genotype not determined.

TABLE 3.—*Utilizing races to identify hybrid plants carrying specific rust-conditioning genes*

| Race no. | Race genotype | | | llmmnn | Reaction[a] of plants possessing genes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | L | $M^3$ | $N^1$ | $LM^3$ | $LN^1$ | $M^3N^1$ | $LM^3N^1$ |
| 108 | $a_La_L$ | $A_M{}^3$ | $A_N{}^1$ | S | S | R | R | R | R | R | R |
| 123 | $A_L$ | $a_M{}^3a_M{}^3$ | $A_N{}^1$ | S | R | S | R | R | R | R | R |
| 52 | $A_L$ | $A_M{}^3$ | $a_N{}^1a_N{}^1$ | S | R | R | S | R | R | R | R |
| 156 | $a_La_L$ | $A_M{}^3$ | $a_N{}^1a_N{}^1$ | S | S | R | S | R | S | R | R |
| 192 | $A_L$ | $a_M{}^3a_M{}^3$ | $a_N{}^1a_N{}^1$ | S | R | S | S | R | R | S | R |
| 154 | $a_La_L$ | $a_M{}^3a_M{}^3$ | $A_N{}^1$ | S | S | S | R | S | R | R | R |

[a] S = susceptible; R = resistant.

rust-conditioning genes identifies the pairs of recessive genes in a rust culture. The homozygosity or heterozygosity of the dominant genes is established by selfing.

*Maintenance of varietal resistance.* — Rust-resistant varieties of cereals and flax often are damaged by new races or by changes in the prevalence of races of the rust fungi that parasitize them. Jensen (8) proposed several methods of varietal diversification as a means to maintain or prolong the usefulness of a variety. Borlaug (2) proposed the use of the backcross method to develop composite varieties of phenotypically similar lines that are genotypically different for resistance.

In 1947, a backcross program was started to develop lines of flax essentially alike except for a single rust-conditioning gene. The variety Bison was selected as the recurrent parent. Except for its susceptibility to North American races of the flax rust fungus, Bison is well adapted for growing in the North Central States. Lines pure for each rust-conditioning gene have been developed from the Bison backcrosses. As 15 of the 32 varieties to which Bison is being backcrossed possess satisfactory resistance to North American races of the flax rust fungus, a wide choice of rust-resistant Bison-like varieties is available. With an ample supply of resistant germ plasm there appears to be no need at the present time to develop composite varieties of flax as Borlaug (2) has suggested for wheat. Should the need arise, however, the lines derived from the Bison backcrosses should serve this purpose.

Although still ample, the reserve of germ plasm for resistance to North American races of the flax rust fungus has been decreasing. During the decade following 1940, races attacking Koto, Victory A, and Newland were discovered. Whether the races attacking these varieties originated as mutations or had merely escaped detection has not been established. Mutations for virulence are less likely to become established on varieties carrying 2 or more genes than on varieties carrying a single gene for rust reaction. Usually virulence is recessive. Therefore, mutant races can attack heretofore resistant varieties only if they possess, as homozygous recessives, the genes for pathogenicity that complement each gene for resistance in a variety. A race may become homozygous for a mutant gene if the identical mutation occurs in both dikaryotic nuclei of a single spore or if haploids carrying an identical mutation unite through sexual reproduction. In either case, the chance of securing 2 or 3 pairs of mutant recessive genes is much less than that of securing 1 pair.

Bison-like varieties possessing nearly any desired combination of genes for rust reaction within the limits of allelism, may be developed by utilizing the selective pathogenicity of natural and hybrid races to identify the resistant genes in the progeny of hybrids of the Bison backcrosses. For example, the $F_1$ of the seventh backcrosses on Bison of Ottawa 770B, Cass, and Polk carrying the *L*, $M^3$ and $N^1$ genes, respectively, have been intercrossed. Each of these genes is independently inherited and conditions high resistance to all known North American races of the flax rust fungus. As shown in Table 3, the $F_1$ plants that were resistant to races 108, 123, and 52, each of which attacked 1 parent but not the other 2, possessed a gene for resistance from each parent. The $F_1$ plants resistant to races 108, 123, and 52 were intercrossed and the progeny inoculated successively with races 156, 192, and 154, each of which attacked plants having a different combination of 2 of the parental genes but not the third. Plants resistant to race 192 possessed the *L* gene, plants resistant to race 156 possessed the $M^3$ gene, and plants resistant to race 154 possessed the $N^1$ gene. Plants resistant to races 156 and 192 possessed the $LM^3$ genes, plants resistant to races 192 and 154 possessed the $LN^1$, and plants resistant to races 156 and 154 possessed the $M^3N^1$ genes. Plants resistant to all 3 races possessed all 3 genes for resistance. Plants susceptible to all 3 races possessed none.

DISCUSSION.—The criterion for degree of rust resistance or susceptibility is the pustule type that develops on the host as a result of infection by a race of the parasite. The criterion for degree of avirulence or virulence of a race of the rust fungus is the pustule type that develops on the host as the result of infection by the parasite. Thus, a single phenomenon is the measure of both pathogenicity of the parasite and reaction of the host. Pustule type is the expression of the interaction of the genic and cytoplasmic complex of the host with the genic and cytoplasmic complex of the parasite as affected by the environment. The inheritance of avirulence and resistance or of virulence and susceptibility, as indicated by pustule type, has been explained in this and earlier studies (4, 5, 6) as the result of specific genes in the host interacting with specific genes in the parasite. While purely speculative, it seems reasonable to assume that obligate parasites, such as the rust fungi, must have evolved in association with their hosts. Granting this, a simple

explanation for the high degree of specificity in the rust fungi and other obligate parasites is to assume that during their parallel evolution, host and parasite developed complementary genic systems.

In addition to facilitating studies on the inheritance of pathogenicity, the development of resistant varieties, and the maintenance of varietal resistance, the use of isogenic lines and differential varieties with single rust-conditioning genes may aid in the solution of other problems concerning host-parasite relations.

Few instances of mutations for pathogenicity in the rust fungi have been reported. This is not surprising when the techniques employed in physiologic race studies and the genetics of host-pathogen interaction are considered. To lessen the possibilities of contamination, uredial cultures often are increased on a variety that differentiates them. As avirulence usually is dominant, a mutant less virulent than the parent is unable to infect that differential variety and is lost. Except when genes for pathogenicity are heterozygous, a mutation toward greater virulence (recessive) can be detected only if simultaneous mutations of identical genes occur in both dikaryotic nuclei of a single spore. Even then, the mutation can be detected only if the differential used possesses a single gene for reaction to that race.

For example, an $F_1$ culture of the cross of race 42 of the flax rust fungus, which attacks all differential varieties except Bombay (13), with race 43, which attacks Bombay only (14), would be heterozygous for all the known genes for pathogenicity. The uredial $F_1$ culture could be propagated indefinitely as a clonal line and any desired quantity of spores produced. The mutation of only 1 dominant gene to its recessive allelomorph would make that spore homozygous for that pair of recessive (virulent) genes and enable it to attack the differential variety whose resistance was conditioned by the complementary gene in the host. By standardizing inoculation techniques, it should be possible to determine the mutation rate of each gene. The plant breeder could then use those flax genes for rust reaction whose complementary rust genes for pathogenicity showed greatest stability.

Resistance to the rust fungi usually has been inherited as a dominant character, and in flax it has been invariably so. Consequently, a test of a variety with a race that attacks only plants having the dominant gene conditioning reaction in that variety would reveal resistant mutants. These may be increased, homozygous lines secured, and the mutant gene identified by the selective pathogenicity of races of known pathogenic genotype. Mutations for loss of resistance may be more difficult to detect. A plant derived from a resistant variety becomes susceptible to a race avirulent on that variety only if both dominant genes conditioning rust reaction mutate to the recessive allelomorphs. Unless the mutation rate is high, a test of the homozygosity of $X_3$ populations to detect single-gene mutants is impractical.

Epidemiology studies have greater meaning and utility if the pathogenic genotype of each isolate is known. Since the new flax rust differentials contain both the genes used in developing resistant varieties and those conditioning resistance in commercial varieties, routine pathological tests inform the breeder of changes in the virulence of races affecting his sources of rust resistance and establishes the pathogenicity of each rust isolate on all commercial flax varieties. Each gene for virulence may be identified, and the screening effect of varieties on the prevalence of genes may be determined.

No satisfactory explanation for physiological or hypersensitive resistance to the rust fungi has been given. Allen (1) has suggested that the physiological bases of resistance and susceptibility will benefit greatly from a rigid control of the genotype of both host and parasite. Flor (6) has noted some of the advantages of using lines developed by the backcross method to study the nature of physiological resistance. These lines are essentially alike except for a single rust-conditioning gene. Nutritional differences between such lines or antigen-antibody reactions between such lines and races of the parasite would more likely be related to rust reaction than similar differences between lines that differ in a large number of genes in addition to the gene for rust reaction.

North Dakota Agricultural College
Fargo, North Dakota

## LITERATURE CITED

1. Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. Ann. Rev. Pl. Physiol. **5**: 225-248.
2. Borlaug, N. E. 1953. New approach to the breeding of wheat varieties resistant to Puccinia graminis tritici. (Abs.) Phytopathology **43**: 467.
3. Flor, H. H. 1942. Inheritance of pathogenicity in Melampsora lini. Phytopathology **32**: 653-669.
4. Flor, H. H. 1946. Genetics of pathogenicity in Melampsora lini. Jour. Agr. Res. **73**: 335-357.
5. Flor, H. H. 1947. Inheritance of reaction to rust in flax. Jour. Agr. Res. **74**: 241-262.
6. Flor, H. H. 1954. Identification of races of flax rust by lines with single rust-conditioning genes. U. S. Dept. Agr. Tech. Bul. 1087, 25 p.
7. Henry, A. W. 1930. Inheritance of immunity from flax rust. Phytopathology **20**: 707-721.
8. Jensen, N. F. 1952. Intra-varietal diversification in oat breeding. Agron. Jour. **44**: 30-34.
9. Kerr, H. B. 1952. Rust resistance in linseed. Nature [London] **169**: 159.
10. Myers, W. M. 1937. The nature and interaction of genes conditioning reaction to rust in flax. Jour. Agr. Res. **55**: 631-666.
11. Stakman, E. C., M. N. Levine, and W. Q. Loegering. 1944. Identification of physiologic races of Puccinia graminis tritici. U. S. Dept. Agr., Agr. Res. Admin., Bur. Entom. and Pl. Quar. E-617, 27 p.
12. Straib, W. 1939. Zum epidemischen auftreten des leinrostes in Ostpreussen. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst **19**: 49-51.
13. Vallega, J. 1942. (1944). Especializacion fisiologica de Melampsora lini in Argentina. An. Inst. Fitotéc. Santa Catalina **4**: 59-74.
14. Waterhouse, W. L., and I. A. Watson. 1944. Further determinations of specialisation in flax rust caused by Melampsora lini (Pers.) Lév. Roy. Soc. N. S. Wales, Jour. and Proc. **77**: 138-144.

# THE RELATION OF VALSA KUNZEI TO CANKERS ON CONIFERS [1]

Alma M. Waterman [2]

SUMMARY

The species of *Cytospora* commonly found on cankers on various species of spruce and of the related Valsa stage were studied in comparison with species of *Cytospora* and of *Valsa* associated with cankers on other coniferous hosts. Pycnidia and perithecia from cankers on *Abies balsamea, Pseudotsuga menziesii, Larix decidua, Tsuga canadensis, Picea abies, P. pungens, P. glauca, P. orientalis, P. rubens, Pinus strobus,* and *P. wallichiana* and from cultures on artificial media and sterilized twigs were studied microscopically. Pycnidial stromata were extremely variable on any 1 host. Characteristics of perithecial stromata were more constant and were used for diagnosis.

The fungus on all coniferous hosts corresponded with *Valsa kunzei,* and on the basis of stromatal variations, descriptions are given of 3 varieties and their corresponding pycnidial stages: *Valsa kunzei* var. *Kunzei* var. nov., typical, on *Abies balsamea, Pseudotsuga menziesii, Larix decidua,* and *Tsuga canadensis; V. kunzei* var. *piceae* var. nov. on *Picea abies, P. glauca, P. pungens, P. orientalis,* and *P. rubens;* and *V. kunzei* var. *superficialis* comb. nov. on *Pinus strobus* and *P. wallichiana.* Cultural characteristics also indicated varietal differences.

Inoculations with isolates of *Valsa kunzei* representing each of the varieties were made on nursery trees of *Picea pungens, P. abies, P. glauca, Pseudotsuga menziesii, Abies fraseri, Larix decidua, Tsuga canadensis,* and *Pinus strobus.* Positive results were obtained only with *V. kunzei* var. *piceae* on *Picea* species and in 3 inoculations on *Pseudotsuga menziesii.*

Experiments in spraying cankered ornamental spruce trees with Bordeaux mixture and in cutting out infected bark indicated that only the latter method, which is not practical, was effective for controlling large cankers.

Among the fungi most commonly reported on trunks and branches of conifers are species of *Cytospora* and associated perfect forms in the genus *Valsa.* These species have been listed as associated with cankers, or on dead branches or trunks, but the parasitism of only a few species has been studied. Cytospora cankers have for some time been observed on species of spruce in the eastern United States. A brief comparative study of this species of *Cytospora* and of an associated species of *Valsa* on spruce with isolates of Cytospora and Valsa from other conifers is reported herein. The degree to which cross infection by these isolates occurs on conifers was determined.

HOSTS AND DISTRIBUTION.—Gilgut (6, 7) reported *Cytospora kunzei* Sacc. as the cause of canker on Colorado blue spruce (*Picea pungens* Engelm.) and Norway spruce (*P. abies* (L.) Karst.) in Massachusetts. This fungus was also reported by the writer in 1937 (15) on *P. pungens* from Vermont, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Maryland, and Minnesota; on *P. abies* from Rhode Island, New York, Pennsylvania, and Illinois; on *P. glauca* (Moench.) Voss from Massachusetts; *P. rubens* Sarg. from Maine; and *P. orientalis* (L.) Link from New York and Pennsylvania.

On other conifers, a few species of *Cytospora* associated with cankers have proved to be pathogenic on trees weakened by unfavorable environment. Zentmyer (19) described a new form of *Cytospora cenisia* Sacc. (*C. cenisia littorialis*) as a pathogen on branches and trunks of *Cupressus sempervirens stricta* Ait. in California. It caused serious injury and death of trees weakened by unfavorable environment. Wright (18) reported *Cytospora abietis* Sacc. as the cause of cankers on forest trees of *Abies concolor* (Gord. & Glend.) Lindl. and of *A. magnifica* A. Murr. in the northern Sierra Nevada, when the trees were weakened by environmental factors. Baxter (2) found cankers on plantation trees of Himalayan pine (*Pinus wallichiana* A. B. Jackson) in the Saginaw Forest, Michigan, on which fruiting bodies of a fungus identified as *Valsa superficialis* Nits. and its Cytospora stage were abundant. Girdling cankers at the nodes caused dieback and death of the trees.

In 1930 a canker was described (1) on European larch (*Larix decidua* Mill.) growing in a poor site in a forest reservation in Saratoga County, New York. From cankers collected by the writer in 1936 from these trees, and from European larches in other localities in New York, Massachusetts, and Pennsylvania, a species of *Cytospora* and an associated species of *Valsa* were isolated and studied.

Studies were made on species of *Valsa* and *Cytospora* isolated from cankers, or from branches killed by girdling cankers, on ornamental, plantation, or forest trees. Isolations were made from *Pseudotsuga menziesii* (Mirb.) Franco in New Hampshire, Vermont, Massachusetts, Pennsylvania, and Washington;

[1] Accepted for publication August 10, 1955.

[2] Pathologist, Forest Insect and Disease Laboratory, Northeastern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, New Haven, Conn. The writer expresses her appreciation to Drs. L. E. Wehmeyer and W. W. Diehl for advice about the mycological phase of the study; to Mr. L. Holm of Uppsala, Sweden, for information about the type material of *Valsa kunzei;* and to Miss Edith K. Cash for the Latin descriptions.

*Abies balsamea* (L.) Mill. in Maine and Vermont; *Tsuga canadensis* (L.) Carr. in New Hampshire and Connecticut; *Pinus strobus* L. in Maine, Connecticut, New York, and Virginia; *P. wallichiana* in Maryland; *Picea abies* in Massachusetts and Connecticut; *P. glauca* in Connecticut; *P. orientalis* in Connecticut and Pennsylvania; *P. pungens* in Connecticut, New York, and New Jersey; and *P. rubens* in Maine and New Hampshire.

SYMPTOMS.—Symptoms of attack by *Valsa kunzei* and the associated Cytospora stage include many characteristics in common for the coniferous hosts studied. Cankers frequently start around the base of small twigs on branches or trunks and develop slowly. On blue, white, and oriental spruce, lower branches are chiefly affected. Trunk cankers are more common on red and Norway spruce and therefore cause more damage. Conspicuous branch and trunk cankers are found on European larch, Douglas fir, balsam fir, and eastern hemlock. Branch cankers are relatively inconspicuous on eastern white pine and Himalayan pine, but nodal trunk infection on these hosts may cause extensive girdling cankers and dieback. Resin flow is commonly associated with the cankered areas. It is particularly conspicuous on all infected spruces, covering the cankered areas and often dripping down the trunk or from one branch to another. Resinosis is usually least conspicuous on balsam fir and eastern white pine. The limits of infection in branch and trunk cankers on spruce are not clearly defined, but on other hosts the bark of the infected areas is depressed, with delimiting marginal callus developing slowly.

Removal of resin and outer bark from cankers on spruce reveals the brown killed areas of inner bark and cambium. Within the dead areas, and especially at the margin, occasional small black Cytospora pycnidia are evident. In moist summer weather, these may exude amber or orange colored spore tendrils. Small black erumpent fruiting bodies of Valsa may appear among the pycnidia, as reported by Marsden (11), or throughout the length of branches recently killed by girdling cankers. The Valsa stage, however, is rarely abundant. On the other hosts, numerous Cytospora pycnidia are scattered throughout the bark of the cankers. Valsa perithecia are sometimes found among the Cytospora pycnidia in the bark of old cankers, but they are more common on dead branches or trunks. Occasionally the pycnidia also are produced on dead portions, particularly on dead twigs of eastern white pine.

Spruces with branch cankers are disfigured by the disease, but the trees are rarely killed. Trunk cankers on red and Norway spruces may eventually girdle the trees, causing their death. Trees of the other coniferous hosts are not seriously affected, except those growing in unfavorable sites or weakened by other environmental factors. On eastern hemlock, cankers rarely form, except around branch stubs on pruned trees in hedge-row plantings.

IDENTITY OF THE SPECIES OF VALSA AND CYTOSPORA.—The morphological characters of the Cytospora stage were not sufficiently distinctive to indicate that more than 1 species was involved. The size and shape of the conidia were similar in the pycnidia on all hosts. The size of the stromata and the size and number of the pycnidial locules in a stroma varied over a wide range on any 1 host. When compared with similar ranges of other hosts, the differences were too slight to be considered as species distinctions.

Marsden (11) reported the isolation, from cankers on *Picea abies* and *P. pungens*, of a Valsa that was identified by Wehmeyer as *Valsa kunzei* (Fr.) Fr. The perithecial stromata of the fungi in the collections from the various coniferous hosts used in the present study were compared closely with those of this fungus. Four other species of *Valsa* have been reported in the United States on 1 or more of these hosts. These species are *V. abietis* Fr., *V. curreyi* Nits., *V. pini* Alb. & Schw. ex Fr., and *V. superficialis* Nits. Of these, only *V. superficialis*, reported on *Pinus strobus* and *P. wallichiana*, seemed to correspond with any of the isolates studied.

Sections of the perithecial stromata in collections from all the hosts showed that the perithecia were surrounded by stromatic tissues and that a more or less pronounced dark marginal zone of tissue delimited the size of the stromata. On certain hosts, particularly balsam fir and white pine, pycnidial stromata sometimes formed immediately adjacent to perithecial stromata. In such cases the marginal line was continuous around the entire stromatic area, including both pycnidia and perithecia. When pycnidial stromata occurred alone, especially in young cankers, the marginal line was usually indistinct or lacking. The character of the perithecial stromata and the location of the delimiting marginal line in the cortex differed sufficiently among the various hosts to be considered indicative of varietal distinctions.

This development of stroma and marginal zone is characteristic of the *Valsa* subgenus *Leucostoma* Nits. (12), which includes *V. kunzei* and *V. superficialis*. Von Höhnel (8) described *Leucostoma* as a genus separate from *Valsa*, on the basis of stromal development. Wehmeyer (17) also considered *Leucostoma* as a genus characterized by perithecia surrounded by stromatic tissue that has a delimiting zone line. The writer has retained the genus name *Valsa*, however, until such time as the relation between it and *Leucostoma* is more definitely established.

The asci and ascospores on all hosts studied were similar and corresponded in form and size range to those described for *Valsa kunzei* and *V. superficialis*. The number of perithecia in a stroma and the shape and size of the perithecia and stromata also were comparatively uniform, but there were slight variations among the different hosts as well as among various collections from the same host.

In *Abies balsamea*, *Pseudotsuga menziesii*, *Larix decidua*, and *Tsuga canadensis*, the marginal zone line

develops before the perithecia become mature. It arises in the innermost layers of the periderm. In a vertical section through the perithecial stromata, this black line is visible with a hand lens and is conspicuous in a microtome section (Fig. 1). The periderm is split by the developing stroma, and the innermost periderm cells become a part of the zone line which curves closely around the bases of the perithecia. The zone line may extend through the periderm for some distance between the separate stromata, curving inward to border a series of several perithecial stromata.

Around the perithecia, the stromatic tissue includes portions of the decomposing bark cells but develops outward into a compact cushion-like stroma that soon becomes penetrated by the necks and the ostioles of the perithecia. Superficially, this stromatic tissue may be noticeable as a grayish-white disc. This form of perithecial stroma corresponds with that of *Valsa kunzei* described by Wehmeyer (16) on *Thuja plicata* Don.

This fungus was originally described by Fries (4) as *Sphaeria kunzei* "in cortice Abietis frequens." He referred to a specimen of it, number 153 in the exsiccati of Schmidt and Kunze (13) and designated as *Sphaeria pini* Alb. & Schw. *α pini*, on bark of *Pinus picea*, which is now known as *Abies alba* Mill. In another reference (3), he stated that the fungus occurred "in cortice Pini piceae; etiam in Abiete," indicating that he had found it on both *A. alba* and *Picea abies*. In 1849, Fries (5) transferred *S. kunzei* to his genus *Valsa*, as *V. kunzei*. In the present study, the fungus on *Abies balsamea* seemed to correspond with the description of the type specimen of *V. kunzei*. Therefore, the fungus in the collections from *A. balsamea*, *Pseudotsuga menziesii*, *Larix decidua*, and *Tsuga canadensis*, from New England, is designated as typical of *V. kunzei*.

On species of *Picea* (*P. abies*, *P. glauca*, and *P. pungens*), the delimiting zone line is relatively indefinite and inconspicuous. It frequently forms as an irregular broken band of tissue in the cortex slightly below the perithecia when they are fully mature (Fig. 2). Because of its variability, the zone line sometimes is not noticeable except by microscopic examination of microtome sections. The area between the zone line and the bases of the perithecia is filled with decomposed cells of the innermost layer of the periderm and closely matted mycelium in and around the cortex cells. Although this form of stroma varies slightly from that on *Abies balsamea* and other hosts, the differences do not seem to be sufficient for designating the fungus as a separate species of *Valsa*. The fungus therefore is considered to be a variety of *Valsa kunzei* on *Picea abies*, *P. pungens*, *P. glauca*, *P. orientalis*, and *P. rubens*, in the collections studied from New England, New York, New Jersey, Pennsylvania, Maryland, and Illinois.

On *Pinus strobus* and *P. wallichiana*, the marginal zone is conspicuous but is usually located irregularly in the cortex some distance below the bases of the perithecia (Fig. 3). Although it is first formed in the

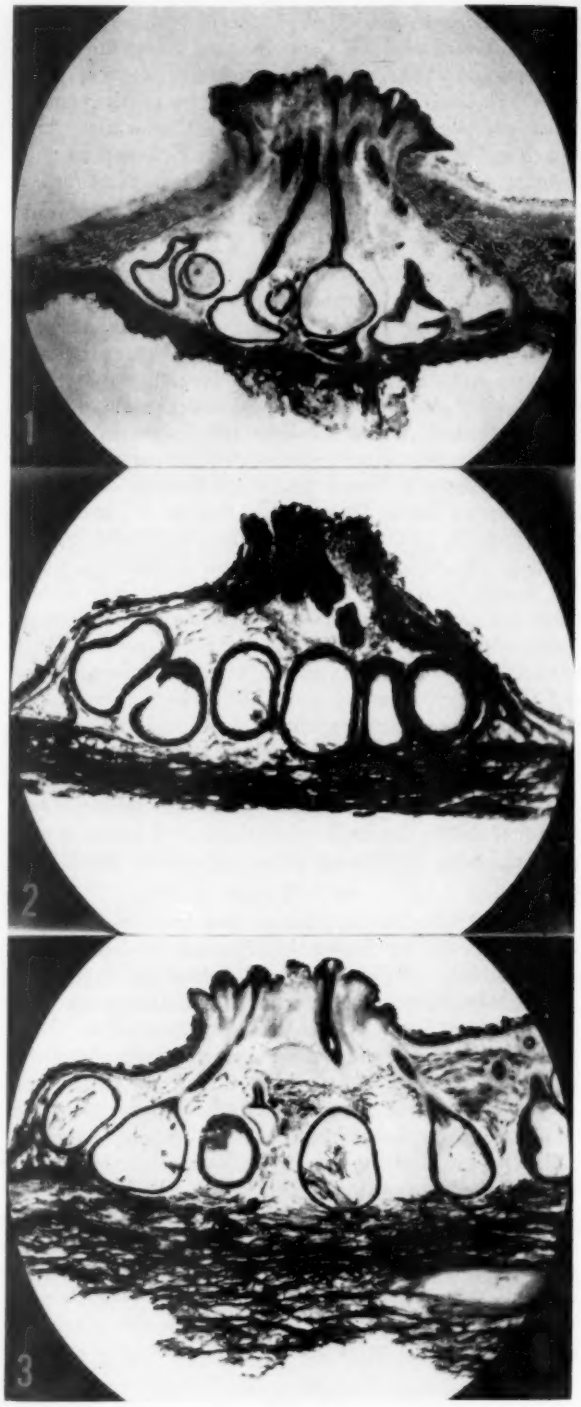



FIG. 1–3.—Fig. 1. *Valsa kunzei* var. *kunzei* on *Abies balsamea*. Young stroma showing black zone line of tissue below perithecia delimiting the size of the stroma. ×75.—Fig. 2. *Valsa kunzei* var. *piceae* on *Picea glauca*. Old well-developed stroma, delimited by wide dark irregular band of tissue. ×75.—Fig. 3. *Valsa kunzei* var. *superficialis* on *Pinus strobus*. Large cushion-like stroma, with narrow black delimiting line of tissue in the cortex. ×75.

periderm, it curves deeply into the cortex as an unbroken line, and the perithecia are formed in the outer cortex. The zone line may extend through the cortex for some distance, bordering the stromata of several clusters of perithecia. A comparison with the perithecia on the other coniferous hosts failed to show any characters of specific significance. The descriptions of *Valsa superficialis* (9, 16) indicate that the fungus in the collections on *Pinus strobus* and *P. wallichiana* in this study corresponds with that species. The location of the marginal line in the cortex, however, is the characteristic by which the stromata in these collections differ from those on the other coniferous hosts studied. Therefore, this fungus also is similar to *Valsa kunzei* and is here described as a variety of that species in collections from New England, New York, Maryland, and Virginia.

It is evident, therefore that there is considerable similarity in the development of the stromata on the various hosts. Slight differences, however, indicate that 3 types of stromal development are involved. These are used as the basis for distinguishing 3 varieties of the species *Valsa kunzei.*

*Valsa kunzei* var. **kunzei** var. nov. (typical)[3].—Synonyms: *Sphaeria pini α pini* Schm. & Kunz. Deuts. Schwamme 7:1-6. No. 153. 1817; *Sphaeria kunzei* Fr. In Kunz. & Schm., Myk. Hefte 2: 45, 1823, also Syst. Myc. 2: 389. 1823; *Valsa kunzei* Fr. Summa Veg. Scand., p. 411. 1849.

Stromata medium to large, scattered, pustulate, with circular grayish disc sometimes inconspicuous, penetrated by small black ostioles; perithecia numerous, oval, with long converging necks, surrounded by stromatic tissue, sometimes with decomposed bark cells in the stroma; wide dark marginal band of tissue limiting the size of the stroma, and separating it from the cortex, curving closely around the bases of the perithecia, sometimes bordering several clusters of perithecia; asci narrow, clavate, sessile, 8-spored, 24–30 $\mu$ × 4–5 $\mu$; ascospores cylindric, curved, hyaline, continuous, 5–9 × 1.5 $\mu$.

Pycnidial stage: *Cytospora kunzei* var. **kunzei** var. nov. (typical).[3] Synonym: *Cytospora kunzei* Sacc., Syll. Fung. 3: 270. 1884. Stromata conoid, solitary or grouped, 1–3 mm at the base, yellowish gray or dark gray disc at the top usually conspicuous, with 1 central opening; multilocular, pycnidial locules irregular in size and shape, more or less radially arranged; dense hymenial layer of filamentous conidiophores; conidia continuous, hyaline, curved, 4–6 × 1 $\mu$; narrow dark marginal band of tissue delimiting pycnidial stromata that are scattered among perithecial stromata; indistinct or lacking when pycnidial stromata occur alone.

[3] The variety designated as *Valsa kunzei* var. *kunzei* (typical) agrees closely with the Latin description of *V. kunzei* Fr. in Saccardo's Syll. Fung. 1: 139, 1882; also, the variety *Cytospora kunzei* var. *kunzei* (typical) corresponds with the Latin description of *C. kunzei* Sacc. in Syll. Fung. 3: 270, 1884.

On *Abies balsamea, Pseudotsuga menziesii, Larix decidua, Tsuga canadensis,* from collections in New England.

*Valsa kunzei* var. **piceae** var. nov.—Stromata magna, solitaria vel aggregata, disco circulari fusco-griseo rare prominenti, ostiolis magnis atris caespitosis perforato praedita; perithecia numerosa, ovalia vel compressa, collis longis convergentibus munita, stromate subevoluto plerumque mycelio laxe intertexto et cellulis corticis disintegrantibus composito, circum colla peritheciorum densiori, cincta; taenia marginali fusca angusta infra bases peritheciorum, interdum intermittens et indefinita, irregulariter in cortice evoluta; asci angusti clavati sessiles octospori 24–30 $\mu$ × 4–5 $\mu$; ascosporae cylindracae curvatae hyalinae continuae 5–9 $\mu$ × 1.5 $\mu$; asci ascosporaeque varietatae typicae similes.

Status pycnidicus: *Cytospora kunzei* var. *piceae* var. nov. Stromata late conoidea, basi 3–4 mm lata, disco fusco-griseo apice ore uno centrali aperienti praedita; multilocularii loculi pycnidici magnitudine formaque variabiles labyrinthiformes plus minusve radiatim dispositi; strato hymeniali denso conidiophorum filamentosorum vestiti; conidia continua, hyaline, curvata, 4–6 $\mu$ × 1 $\mu$; taenia texturae marginali angusta fusca stromatem limitanti in cortice infra loculos pycnidicos intimos interdum evoluta, plerumque intermittenti et indefinita. A varietate typica stromatibus latioribus et taenia marginali irregulari indefinite differt.

Stromata large, solitary or grouped, circular grayish disc rarely prominent, ostioles large, black clustered; perithecia numerous, oval, or flattened by crowding, with long converging necks; surrounded by poorly developed stroma, usually composed of loosely matted mycelium and decomposing bark cells; merging into denser stromal tissue around perithecial necks; narrow dark marginal band of stromatic tissue developed irregularly in the cortex slightly below the bases of the perithecia, sometimes intermittent and poorly defined; asci narrow, clavate, sessile, 8-spored, 24–30 $\mu$ × 4–5 $\mu$; ascospores cylindric, curved, hyaline, continuous, 5–9 $\mu$ × 1.5 $\mu$; asci and ascospores resembling those of the type variety.

Pycnidial stage: *Cytospora kunzei* var. **piceae** var. nov. Stromata broadly conoid, 3–4 mm at the base, dark gray disc at the top with 1 central opening; multilocular, pycnidial locules irregular in size and shape, labyrinthiform, more or less radially arranged; dense hymenial layer of filamentous conidiophores; conidia continuous, hyaline, curved, 4–6 $\mu$ × 1 $\mu$; narrow dark marginal band of tissue delimiting stroma occasionally developing irregularly in the cortex slightly below the innermost pycnidial locules, usually intermittent and poorly defined. Variety differs from the type variety in the broader stromata and the poorly defined irregular marginal band of tissue.

On *Picea abies, P. glauca, P. pungens, P. orientalis, P. rubens,* from collections in New England, New York, New Jersey, and Pennsylvania.

Specimens of perithecial and pycnidial stages on

*Picea glauca*, Morris, Conn., have been deposited in the National Fungus Collections, Beltsville, Maryland.

*Valsa kunzei* var. *superficialis* comb. nov. — Synonym: *Valsa superficialis* Nits. Pyren. Germ. p. 232. 1870. Stromata large, solitary or confluent, circular yellowish-grey disc around erumpent ostioles which are large, black, and clustered; perithecia numerous, oval or flattened by crowding, with long necks surrounded by dense stromatic tissue; narrow dark continuous irregular marginal band of tissue forming at first in the periderm, but curving deeply into the cortex below the perithecia, frequently bordering the stromata of several clusters of perithecia; tissue between marginal line and bases of perithecia composed of mycelium and decomposing bark cells; asci and ascospores as in type variety.

Pycnidial stage: *Cytospora kunzei* var. *superficialis* comb. nov. Synonym: *Cytospora superficialis* Höhn. Mitt. Bot. Tech. Hochsch. Wien 3: 5-15. 1928. Stromata broadly conoid, 4–6 mm at the base, yellowish-gray disc at the top usually conspicuous, with one central opening; multilocular, pycnidial locules irregular in size and shape, labyrinthiform; dense hymenial layer of filamentous conidiophores; conidia continuous, hyaline, curved, 4–6 $\mu \times 1\ \mu$; narrow black marginal band of tissue in the cortex delimiting the pycnidial stromata frequently present, particularly when perithecial and pycnidial stromata occur in proximity.

On *Pinus strobus* and *P. wallichiana* from collections in New England, New York, Maryland, Virginia, and Michigan.

Cultural study.—In the cultural study of the Valsa and Cytospora stages, isolates from the various hosts (Table 1) were grown on agar media and on sterilized twigs of the same host or a closely related species. In certain isolates of Valsa, the ascospores could not be separated readily from one another for monospore cultures. Monoascus cultures, therefore, were made from all Valsa collections. All Cytospora isolations were from monoconidia. The cultures from the Valsa asci resembled closely those from the Cytospora conidia when both stages were present in the same collection. When only the Valsa or the Cytospora stage was present in a collection, the isolates were carefully studied to determine whether all isolates from a host were identical in growth characters.

Mature Cytospora pycnidia developed in all agar cultures, but no perithecia were formed. In cultures on Leonian's medium (10) pycnidia developed freely, with distinct and constant characteristics according to the hosts of the original isolates. These cultures, therefore, were the basis for determining the cultural characteristics of the 3 varieties of *Valsa kunzei* and the relation of the Cytospora stage on a canker to the Valsa stage on dead branches. On sterilized twigs in culture flasks with high humidity, the pycnidia were more stromatic and variable than those formed in nature. When the twig cultures had partially dried out, perithecia also were developed by some of the isolates.

In agar cultures, the pycnidial stromata of *Valsa kunzei* var. *kunzei* and *V. kunzei* var. *superficialis* were relatively small, fairly numerous, white to gray or black with white hyphae at the top. On sterilized twigs, these varieties produced both pycnidial and perithecial stromata with the dark marginal zone line characteristically located in the cortex, delimiting both types of stromata.

In agar cultures of *Valsa kunzei* var. *piceae*, the pycnidial stromata were olivaceous to black, rare, but very large. Similar pycnidial stromata developed on sterilized twigs. The dark brown marginal line was formed occasionally in the cortex slightly below the stromata. No perithecial stromata developed.

Inoculations. — The relation of the Cytospora isolates from spruce with those from other conifers was tested by inoculations on nursery trees of 8 species of conifers, including *Picea pungens*, *P. abies*, *P. glauca*, *Pseudotsuga menziesii*, *Larix decidua*, *Pinus strobus*, *Tsuga canadensis*, and *Abies fraseri* Poir.

The inoculum consisted of mycelium and mature Cytospora pycnidia from agar cultures obtained by isolations from *Picea abies*, *P. pungens*, *P. rubens*, *Abies balsamea*, *Larix decidua*, *Pseudotsuga menziesii*, *Pinus strobus*, and *Tsuga canadensis*. All inoculations were made by cutting with a sterilized scalpel 2 sides of a small triangular section of bark and cambium on a branch. The point of this section was turned back from the wood sufficiently to allow the insertion of a

Table 1.—*Origin of the isolations used in the cultural study of Valsa kunzei on coniferous hosts*

| Host | Stage of fungus isolated from each collection | | |
|---|---|---|---|
| | Both Valsa and Cytospora | Valsa only | Cytospora only |
| *Abies balsamea* | Me.-1[a] | Me.-2, Vt.-1 | |
| *Larix decidua* | Mass.-1, N.Y.-1 | | |
| *Pseudotsuga menziesii* | N.H.-1 | Mass.-1 | Vt.-1 |
| *Tsuga canadensis* | | N.H.-1 | Conn.-1 |
| *Picea pungens* | Conn.-1 | | N.Y.-1, N.J.-1 |
| *Picea abies* | | Mass.-1 | Conn.-2, Mass.-1 |
| *Picea glauca* | | | Conn.-1 |
| *Picea orientalis* | | | Conn.-1, Pa.-2 |
| *Picea rubens* | | | Me.-2, N.H.-2 |
| *Pinus strobus* | N.Y.-1 | Me.-2, Conn.-1 | Va.-1 |
| *Pinus wallichiana* | | | Md.-1 |

[a] Numerals indicate number of collections studied from each state.

small piece of inoculum. The bark was then pressed back against the inoculum, and the part inoculated was wrapped with wet cotton and waxed paper. A total of 288 inoculations were made in the spring on the various conifers. Simulated inoculations were made on check trees in the same manner but with the insertion of sterile agar. The wrappings were removed after 2 weeks. During the growing season, all check trees healed with callus and showed no indication of infection.

An inoculation was considered to be positive only if a canker was formed and an organism resembling that of the original inoculum was reisolated. In a few inoculations, considerable callus was produced around the wound and the fungus could not be reisolated. Such results were rated as negative.

Positive results were obtained only from inoculations with isolates of *V. kunzei* var. *piceae* from *Picea abies*, *P. orientalis*, and *P. glauca* on *Picea* species and on *Pseudotsuga menziesii* (Table 2). Characteristic cankers with excessive resin exudate and Cytospora fruiting bodies were obtained on the species of spruce and from 3 inoculations on Douglas fir with the isolate from *Picea pungens*. In 7 other similar inoculations on Douglas fir, small cankers developed with resin exudate but soon became callused and healed over in a year's time. The fungus could not be reisolated from these inoculations. No perithecia were formed in association with the pycnidia on any of the cankers, therefore no determination of the variety of *Valsa kunzei* could be made from the host. Cultures of the isolated organism, however, resembled those of *V. kunzei* var. *piceae*, rather than of *V. kunzei* var. *kunzei*.

Infection of conifers by species of *Cytospora* has been generally considered to occur only on trees weakened by some other factor, such as unfavorable site, drouth, frost, or fire injury. Many of the trees from which the collections were made had been subjected to these or similar adverse conditions. In contrast, the trees used for the inoculations were young healthy trees, most of them growing in a favorable site. Therefore, the vigor of the trees might explain the failure of most of the inoculations. The Cytospora from spruce, however, seems to be a more active wound parasite on its specific hosts, and also may infect Douglas fir.

CONTROL OF CYTOSPORA ON SPRUCE.—The prevalence of Cytospora cankers on ornamental blue spruces and the unsightliness of affected trees has caused considerable demand for an efficient method of control (14). Several methods were tried experimentally.

All dead branches were removed from 10 diseased trees of white and blue spruce in a large planting. In April 1938, the Cytospora cankers on living branches were treated as follows: 1) thirteen large cankers on 6 trees were painted with a thick coat of a paste made of Bordeaux mixture and linseed oil; 2) two cankers on another tree were scraped to remove the outer bark in which the fruiting bodies were imbedded (no Bordeaux paste was applied) 3) three cankers on 2 trees were treated by carefully cutting out all bark and cambium in the cankered area and scraping off the margins of the cankers (no Bordeaux paste was applied) and 4) 1 canker was treated as in method 3 and painted with Bordeaux paste.

In the spring of 1939, 7 of the 13 branches given treatment 1 had died, with fruiting bodies of Cytospora and Valsa abundant under the paste and beyond the treated areas. One canker of the 2 given treatment 2 had girdled the branch, causing its death. All branches given treatments 3 and 4 were healthy, with no evidence of the spread of the fungus. Callus was rapidly covering the exposed wood. In 2 of the 3 cankers given treatment 3, without the Bordeaux paste, the callus eventually completely covered the treated areas and the branches remained healthy. All branches treated by methods 1 and 2 died during the summer of 1940, but those treated by methods 3 and 4 were still alive.

A second series of cankers on 6 additional trees was treated in June, 1941, by cutting out the bark and cambium of the affected areas. One canker per tree was selected, on a branch approximately 4 in. in circumference at the canker. The lengths of the cuts required to remove infected bark were 19, 10½, 6½, 6, 5½, and 3½ in. The widths of the cuts at the widest part were 2, 1½, 1¼, 2¼, 1½, and 1 in., respectively. Rapid callus formation occurred in all cases and the branches remained alive into the following growing season. No further observations were made until the spring of 1946 when the smallest treated canker was entirely healed and the branch was still healthy. Cankers on the remaining branches were partially callused, but new infections between the treated areas and the branch tips had caused girdling cankers.

In May, 1941, 4 infected trees with a few dead or cankered branches were selected for spray experiments. After the dead or noticeably infected branches were pruned out, all remaining small-twig cankers were tagged. One tree was left untreated and the others were sprayed thoroughly with a 4-4-50 Bordeaux mixture, to which casein had been added as a spreader. The spray was applied particularly to the branches, from the trunk outward. Two additional applications were made at 2-week intervals. By the following spring, the small cankers had increased in size and new infections were present on both the treated and untreated trees.

TABLE 2.—*Results of inoculations on species of Picea and on Pseudotsuga menziesii with isolates of Valsa kunzei var. piceae*

| Species inoculated | Infections[a] obtained with isolates from indicated sources | | |
|---|---|---|---|
| | Picea abies | Picea orientalis | Picea pungens |
| *Picea abies* | 1/3 | 5/9 | 9/14 |
| *Picea glauca* | 2/2 | | 10/13 |
| *Picea pungens* | 2/2 | | 13/13 |
| *Pseudotsuga menziesii* | 0/2 | 0/1 | 3/13 |

[a] Numerator is number of infections; denominator is number of inoculations.

It is evident that none of the above methods proved satisfactory or practical for the control of the disease. In the case of small individual branch cankers, infection may be controlled by cutting out the affected bark, but in most cases this method would be difficult and impractical.

FOREST INSECT AND DISEASE LABORATORY
NORTHEASTERN FOREST EXPERIMENT STATION
FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. ANON. 1930. Larch canker. N. Y. Conserv. Dept. Rpt. 20: 204.
2. BAXTER, D. V. 1937. Development and succession of forest fungi and diseases in forest plantations. Univ. Mich. School Forestry and Conserv. Cir. 1, 44 p.
3. FRIES, E. M. 1822-1823. Systema mycologicum. v. 2. Berlingiana, Lund. 2 sect., 620 p.
4. FRIES, E. M. 1823. Synopsis scleromycetum in Suecia nuper detectorum, praemissa nova Sphaeriarum dispositione. In Kunze and Schmidt, ed., Mykologische hefte **2**: 41-60.
5. FRIES, E. M. 1846-1849. Summa vegetabilium Scandinaviae. Holmiae and Lipsiae, Upsala. 2 sect., 572 p.
6. GILGUT, C. J. 1936. Cytospora canker of spruces. Natl. Shade Tree Conf. Proc. **12**: 113-118.
7. GILGUT, C. J., AND O. C. BOYD. 1933. Cytospora canker of Picea spp. (Abs.) Phytopathology **23**: 11.
8. HÖHNEL, F. VON. 1918. Mykologische Fragmente. Ann. Mycol. **16**: 35-174.
9. HÖHNEL, F. VON. 1928. Über de für Europa beschriebenen Cytosporaarten der Abietineen. Mitt. Bot. Inst. Tech. Hochsch. Wien. **5**: 5-15.
10. LEONIAN, L. H. 1924. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. **11**: 19-50.
11. MARSDEN, D. H. 1948. A Valsa associated with Cytospora canker of spruces. Phytopathology **38**: 307-308.
12. NITSCHKE, T. 1870. Pyrenomycetes Germanici. Bd. 1, Lief. 2. Edward Trewendt, Breslau. 320 p.
13. SCHMIDT, J. C., AND G. KUNZE. 1817. Deutschlands Schwamme in getrockneten Exemplaren **7**: 1-6, nos. 151-175.
14. STRONG, F. C. 1953. Spruce branch canker. Natl. Shade Tree Conf. Proc. **29**: 30-35.
15. WATERMAN, ALMA M. 1937. Cytospora canker of spruce. U. S. Dept. Agr. Pl. Dis. Rptr. **21**: 55.
16. WEHMEYER, L. E. 1924. The perfect stage of the Valsaceae in culture and the hypothesis of sexual strain in this group. Mich. Acad. Sci., Arts, and Letters. Papers **4**: 395-412.
17. WEHMEYER, L. E. 1926. A biologic and phylogenetic study of the stromatic Sphaeriales. Amer. Jour. Bot. **13**: 575-645.
18. WRIGHT, E. 1942. Cytospora abietis, the cause of a canker of true firs in California and Nevada. Jour. Agr. Res. **65**: 143-153.
19. ZENTMYER, G. A. 1941. Cytospora canker of Italian cypress. Phytopathology **31**: 896-906.

## REPORT AND ABSTRACTS OF THE THIRTY-SEVENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The thirty-seventh annual meeting of the Pacific Division of the American Phytopathological Society was held on the California Institute of Technology campus in Pasadena, California, June 22 to 24, 1955, in conjunction with the annual meeting of the Pacific Division of the A. A. A. S. Included on the program were 28 papers and a tour of biological research facilities at California Institute of Technology, including the Earhart Plant Research Laboratories. The Phytopathologists' Dinner on Wednesday evening, June 22, was attended by 92 members and guests. Dr. G. W. Keitt was guest speaker.

The membership voted unanimously to hold the next meeting of the division in conjunction with the Pacific Division of the A.A.A.S., meetings in Seattle June 15 to 20, 1956.

Officers elected for the year 1955–56 were *President*, S. M. DIETZ, Oregon State College, and *Vice-President*, WILLIAM NEWTON, Saanichton, British Columbia. EARLE C. BLODGETT, *Councilor*, and ROY A. YOUNG, *Secretary-Treasurer*, have 1 more year of a 2-year term.

ROY A. YOUNG, *Secretary-Treasurer*

## ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

***Translocation of streptomycin and tetracycline in cuttings of Pyracantha.*** ALCORN, STANLEY M., AND PETER A. ARK. Ten-inch cuttings of *Pyracantha augustifolia*, Schneid., the bottom 2 in. of which were defoliated, were immersed approximately 1 in. in test antibiotic solutions for different exposure periods, after which they were needle inoculated at the tip with the fireblight organism, *Erwinia amylovora*, and incubated for about 1 week. The average length of blighted tissue in inches was used as an indication of the degree of translocation. The ratios of the

average lengths of blighted tissue of shoots treated with 1:10,000 tetracycline to those of nontreated shoots were 0.63/6.98, 0.025/3.86, 0/6.26, 0/6.73, and 0/5.15 for 4, 6, 18, 42, and 65 hours exposures, respectively. For the same exposure period the ratios of shoots treated in 1:10,000 streptomycin were 7.4/7.8, 1.9/2.88, 6.49/7.4, 3.42/7.0, and 5.2/6.88, respectively. When 1:10,000 tetracycline was used for shorter periods of ¼, ½, 1, and 2 hours, infection ratios of treated to nontreated cuttings were 4.79/7.91, 3.16/7.91, 3.37/7.91, and 2.26/7.91, respectively. When shoots were exposed to 1:20,000, 1:50,000, and 1:100,000 tetracycline for 4 hours, the infection ratios were 1.61/7.62, 2.53/7.62, and 2.95/7.62, respectively, whereas 6-hour exposure periods gave infection ratios of 0.12/2.88, 0.35/2.88, and 1.07/2.88, respectively.

*Propagation from scales of treated croft lily bulbs.* BALD, J. G., AND PHILIP A. CHANDLER. A large scale propagation experiment was made to obtain pathogen-free stocks of Croft lily for inoculation experiments and for comparison in field trials with commercially grown lilies. After preliminary trials of propagation methods and fungicidal treatments, 23 bulbs were cured at 95°F and stored at room temperature for some weeks. After a 2-day presoaking in cold water, they were immersed in hot water containing 1:200 formaldehyde for 2 hours at 115°F, then soaked in in 1:1000 Puratized Agricultural spray (7.5 per cent tris(2-hydroxyethyl)(phenylmercuri)amonium lactate) for 24 hours. Thirty to 50 scales were removed from each bulb, dusted with ferbam, and propagated in a 75°F cabinet in flats of vermiculite. Four successive lots of bulblets, totaling 5860 from 1000 scales, were removed from the cabinet, and the first 3500 were planted in small pots of sterilized soil. As far as could be determined, the treatments eliminated pathogenic nematodes and all fungi causing lesions on bulbs or roots, except *Fusarium* sp. and a Phycomycete. These organisms were associated with characteristic small, reddish brown, sunken root lesions on many plants and with typical Fusarium basal rot symptoms on 3 bulbs. The characteristic root rot of lilies forced in commercial greenhouses did not appear on the bulblet plants.

*Application of hot-water treatment to growers' lots of gladiolus cormels.* BALD, J. G., AND BRIAN B. MARKLEY. The adaptation of hot-water treatment of gladiolus cormels from experimental conditions to growers' practice involved no essential changes of principle. There were some advances in technique. Control of Fusarium and other diseases was attained on a field scale. For successful treatment, cormels had to be harvested from warm soil after growth during summer. The best pretreatment was the curing of the cormels with the parent corms immediately after harvest for 1 week or longer at 95°F. Hot-water treatment improved rather than diminished germinability when applied before root initials began to appear. Cormels were presoaked for 2 days in water at shed temperature. For the last 2–5 hours of this period, they were held in a 1:200 aqueous dilution of commercial 38 per cent formaldehyde. Final treatment consisted of immersion of mesh containers, ⅔ filled with cormels, in water at 135°F for 30 minutes. Immediately after removal from the bath, the cormels were cooled rapidly and thoroughly dried. Cool storage for 5–6 weeks after hot-water treatment helped to improve germinability. Gladioli that responded best to treatment included the majority of widely grown varieties that are most susceptible to Fusarium disease, particularly to basal rot.

*Nematophagus fungi in citrus orchards.* DEWOLFE, T. A., L. J. KLOTZ, AND S. HASHIMOTO. Nematophagus fungi were found during an investigation of the flora of a wood shavings mulch in a citrus grove watered by sprinklers. A great increase in the growth and fruit production of the trees followed the establishment of the mulch-sprinkler practice. To account in part for this improvement, the following theory is suggested. In nature, nematophagus fungi have developed under conditions of natural rainfall and mulch. Furrow irrigation and clean cultivation, as practiced in some California citrus groves, do not represent natural conditions and therefore are not favorable for nematophagus fungi to maintain themselves at their maximum efficiency. Because of this, citrus nematodes are able to increase and to affect adversely the yield of citrus trees. Conversely, since sprinkler irrigation and artificial mulches do approximate natural conditions, they are favorable for the increase of nematophagus fungi that reduce the nematode population, thereby bringing about an improvement of tree health and fruit yield.

*The effect of $CO_2$ on the vertical distribution of various strains of Rhizoctonia solani.* DURBIN, RICHARD D. Strains of *R. solani* were found that may preferentially attack different parts of a plant: aerial portions, the crown near the soil surface, or roots 3 in. or more below the surface. Fifty-nine clones were arbitrarily classified into these 3 groups according to the part from which they were isolated. Their tolerance to $CO_2$ was studied by observing the effect of 20 per cent $CO_2$ on linear growth rate in a flowing gas system in which the oxygen was maintained at 20 per cent. The mean percentage inhibition, as compared with growth in flowing air, was 77 for 16 aerial isolates, 51 for 17 surface isolates, and 40 for 26 subterranean isolates. Levels of $CO_2$ as low as 1–3 per cent inhibited the linear growth rate of aerial isolates much more than they inhibited subterranean ones. It is concluded that intolerance to $CO_2$ is one of the factors that determine the vertical distribution of various clones of *R. solani* in the host environment.

*The masking of soil treatment effects from use of contaminated planting stock.* FERGUSON, JOHN, AND BRIAN B. MARKLEY. Results from methyl bromide fumigation of gladiolus land emphasize the need of using pathogen-free stock to analyze the effects of treatments for control of soil-borne pathogens. Control of *Rhizoctonia solani*, *Stromatinia gladioli*, and *Fusarium oxysporum* f. *gladioli* by the use of 2, 3, and 4 lb. of methyl bromide per 100 sq. ft. was reflected in increased size of gladiolus corms and lower disease incidence when hot-water-treated planting material was used. The use of the grower's infested corms, on the other hand, gave the anomalous result of poorest yield in soil treated with the higher dosages, whereas the least disease loss appeared in the untreated plots. These results indicate that soil treatment can be expected to give the greatest improvement when used with highly susceptible pathogen-free stock, but should improve growth of less susceptible pathogen-free varieties. Treatment of soil may give no improvement, or may even result in yields lower than those of untreated plots, when used with infested stock. These effects reveal the possible dangers from soil treatments that reduce the competing microflora, resulting in increased disease loss when infested stock is planted.

*A latent virus in a potato clone resistant to virus X and its relation to potato virus S.* GOLD, A. HERBERT, AND JOHN W. OSWALD. Rod-shaped particles occurring in the potato clone USDA 41956, immune from potato virus X, were studied with the electron microscope and found to be approximately 16 mμ in diameter, with a mean length of 677 mμ (S.E. = 10.8 mμ). The mean length of virus X rods from infected *Nicotiana glutinosa* was 520 mμ, distinctly shorter than the 41956 particles. The particles were not found in potato seedlings grown from true seed. Rod-shaped particles were found in seedlings 15 days after mechanical inoculation with juice from clone 41956. Transmission attempts to hosts other than potato were not successful. Antiserum produced by injecting rabbits with juice of clone 41956 reacted with juice of clone 41956, with that of commercial varieties White Rose and Russet Burbank, and with that of inoculated potato seedlings containing rod-shaped particles, but not with that of noninocu-

lated potato seedlings. Antiserum produced in Holland against potato S virus reacted in a manner identical with that of the antiserum against 41956. It is concluded that potato clone 41956 and the commercial varieties tested are infected with a latent virus that is related to or identical with potato S virus.

*Electron microscopy of particles associated with sugar cane mosaic.* GOLD, A. HERBERT, AND J. P. MARTIN. Juice of sugar cane plants infected with sugar cane mosaic, shipped from Hawaii to Berkeley in a frozen state, was found to contain rod-shaped particles 15 mμ in diameter and somewhat variable in length, with lengths most frequently between 620 mμ and 670 mμ. These rods were not found in juice of healthy cane. Later, diseased cane found in San Francisco markets was found to contain rod-shaped particles of the same size. Healthy corn (*Zea mais* L.) contained no such particles, but Golden Bantam Corn inoculated from diseased cane developed symptoms and contained rod-shaped particles of the same size as those of diseased cane. Healthy cane from Louisiana contained no rod-shaped particles, but cane from Louisiana infected with strains A, B, C, and D each contained particles of the same size as did diseased cane from Hawaii and California. The virus is unstable and low in concentration. Partial purification has been obtained. Infectivity is associated with the presence of the particles.

*Evidence that celery is a host of peach yellow leaf roll virus.* JENSEN, D. D. To test possible transmission of peach yellow leaf roll virus through the egg of the vector *Colladonus geminatus*, infective leafhoppers were caged on celery plants, where they deposited eggs. Leafhoppers of the $F_1$ and $F_2$ generations transmitted virus to 26 of 35 peach trees. These results could have either or both of the following explanations: 1) transovarial virus passage in the vector, and 2) celery, if a host of the virus, may have become infected when fed upon by viruliferous leafhoppers during the oviposition period and then provided virus for leafhoppers hatching on celery. The first possibility is not yet excluded. The second explanation is supported by experiments in which virus was recovered by nonviruliferous leafhoppers from 6 celery plants fed on 6 weeks previously by infective leafhoppers. Virus was transmitted from celery to 17 of 23 peach trees. All 50 control trees have remained healthy after having been fed on by groups of 50 leafhoppers taken from the nonviruliferous stock colonies used in these experiments.

*Transmission of the green valley strain of cherry buckskin virus by means of leafhoppers.* JENSEN, D. D. AND H. EARL THOMAS. The Green Valley (California) strain of cherry buckskin (western X) virus was transmitted from infected Royal Ann and Chapman cherry on both Mahaleb and Mazzard root to 115 of 190 Lovell peach seedlings by the leafhopper *Colladonus geminatus* (Van Duzee). The virus was acquired by adults caged on the inoculum trees for approximately 1 month and also by first and second instar nymphs that hatched from eggs deposited in the leaves of a diseased tree. The symptoms of the virus on peach seedlings in the greenhouse were indistinguishable from those caused by the Napa Valley strain of virus and in many cases could not be distinguished from the symptoms of peach yellow leaf roll virus. Trees infected during 1954 were consistently slower in developing second season symptoms in the greenhouse than were those infected with yellow leaf roll virus.

*Swarming of water molds that parasitize citrus.* KLOTZ, L. J., AND T. A. DEWOLFE. *Phytophthora citrophthora* and *P. parasitica* are 2 fungi that, together or separately, cause citrus fibrous root rot, crown rot, or fruit rot. They are common in citrus soils and are spread in irrigation water in the zoospore stage. *Pythium aphanidermatum* causes a damping-off of citrus seedlings where soil temperatures are high and irrigation of seedbeds is by flooding. The fungus reaches seedlings in flooded seedbeds in the zoospore stage. A 16-mm photomicrographic motion picture illustrated the formation and discharge of zoospores of these 3 fungi. Sporangia of both *Phytophthora citrophthora* and *P. parasitica* containing fully formed zoospores were shown discharging zoospores. Active motility, encystment, and germination of these spores were shown. Sporangia stained with 0.005 per cent aqueous neutral red (Color Index number 825) were seen discharging active stained zoospores. Inflated filamentous sporangia of *Pythium aphanidermatum* and vesicles, containing amorphous protoplasm, that have formed from the contents of these sporangia were illustrated. These were succeeded by illustrations of vesicles containing fully formed writhing zoospores that burst through vesicle walls and became free-swimming zoospores.

*The occurrence of Phytophthora and Pythium species on roots of native plants in northern California and southern Oregon.* MIDDLETON, JOHN T., AND DOW V. BAXTER. Roots of plants in their native habitat were recovered from soil, washed in water, surface sterilized, and placed in a variety of agar subtrates containing sterilized plant debris. Recoveries of Phytophthora and Pythium were made from 45 species in 20 families representing herbaceous materials such as Darlingtonia and Mimulus and woody material such as Arctostaphylos, Pinus, Sequoia and Taxus. The species isolated were *Phytophthora cactorum*, *P. cryptogea*, *P. cinnamomi*, and *P. parasitica*, and *Pythium acanthicum*, *P. aphanidermatum*, *P. irregulare*, *P. rostratum*, *P. spinosum*, *P. ultimum*, and *P. vexans*. The recovery of some of these species from native vegetation in its natural habitat demonstrates the presence of these organisms in plant material not associated with man's activity.

*Physiologic leaf spot of Philodendron hastatum.* MUNNECKE, DONALD E. Leaves of *Philodendron hastatum* often have exuding spots, frequently necrotically ringed, that appear to be of bacterial origin. Bacteria are localized between host cells in restricted areas beneath stomates. The spots and sooty molds growing on their exudate often make plants unsalable. Spotting is rare in greenhouses in the cool San Francisco area and prevalent near warmer Los Angeles. Spots increased on plants grown at 70–100°F as compared to plants grown at 55–85°F. Many types of bacteria, isolated from spots, were used to inoculate plants by spraying, by hypodermic injections of leaf blades, petioles, or unfurled leaves, or by vacuum impregnation. All results were negative. Agri-mycin (streptomycin; oxytetracycline), copper, and carbamate sprays were ineffective in control. Thrice-weekly swabbing of leaves with water controlled the disease but is not feasible commercially. Best control was obtained by growing plants with moderate fluctuations of day/night temperatures and by spraying biweekly with captan to control sooty molds. On the basis of controlled laboratory and commercial greenhouse experiments and field observations, it is concluded that the disease is caused by saprophytic bacteria that colonize plant exudations stimulated by high fluctuating temperatures (cool nights, hot days). Products of the bacteria kill adjacent cells.

*Interference between the viruses or virus strains causing yellow leaf roll and western-x disease of peach.* NYLAND, GEORGE. One-year-old Lovell peach seedlings were inoculated concurrently with buds from shoots showing symptoms of peach yellow leaf roll and with buds of shoots with western-x disease. When the yellow leaf roll buds were placed in the main trunk of the trees immediately above the western-x disease buds, severe yellow leaf roll symptoms developed throughout the tops of the trees the following seasons. Two of the 5 inoculated trees were dead by the third year. Trees of this group were essentially similar to those inoculated with yellow leaf roll virus alone. Trees inoculated with the western-x disease virus in the upper

position did not show symptoms of either disease the following year. The second year after inoculation, a few shoots produced near the ground line from 2 of the 5 trees showed symptoms of western-x disease. The third year, 4 trees of this group expressed symptoms. The appearance of these trees was essentially the same as those inoculated with the western-x disease virus alone. These trees were only slightly smaller than the noninoculated checks. Under the conditions of this experiment, only the disease represented by the upper buds appeared in the above-ground portions of the trees.

*Effects of cycloheximide on powdery mildew of grapes and brown rot of peach fruits in the laboratory.* OGAWA, JOSEPH M., AND CLAUDIO VERGARA. Cycloheximide was tested for its protective, systemic, and eradicative action against powdery mildew on young plants of *Vitis vinifera* L. var. Carignane. Sprays containing either 0.5 or 2 ppm cycloheximide plus 1 per cent each of methyl cellosolve and "Carbowax" Compound 4000 were used. In addition, a 2.5 per cent Attaclay dust formulation was tested. Rapidly growing shoots were sprayed or dusted and inoculated 1 day later, and thereafter every 2 days for 5 weeks, with conidia of *Uncinula necator* (Schw.) Burr. The parts treated with the dust or sprays were practically free of infection, whereas new growth and the controls were severely diseased. Only slight necrosis was observed at 2 ppm. This concentration was highly eradicative but caused slight injury to the infected areas. The dust was ineffective as an eradicant and produced shot-holing of young leaves. Cycloheximide at 100 ppm showed excellent protection against the brown-rot fungus (*Sclerotinia fruticola* (Wint.) Rehm) on cling peach fruits. In the eradicant tests, fruits dusted with conidia, incubated in a saturated atmosphere for 1 day for development of infection, and then sprayed with 50 ppm of cycloheximide remained normal, whereas all control fruits developed typical brown rot.

*The barley yellow dwarf virus disease on cereal crops in The Netherlands.* OSWALD, JOHN W., AND T. H. THUNG. Numerous volunteer oat and barley plants exhibiting symptoms of the barley yellow dwarf virus disease were observed in September, 1953, in eastern Holland. Four aphid species known to be vectors of this virus were found in infected volunteer fields—*Macrosiphum granarium, Rhopalosiphum prunifoliae, Rhopalosiphum maidis,* and *Macrosiphum dirhodum.* Trials with the aphids R. *prunifoliae* and *M. granarium* were successful in transmitting the virus from naturally infected barley and oats to both Dutch and American varieties of barley and oats. Under reduced light conditions of the winter months, severe stunting of inoculated plants occurred, but little change in pigmentation developed. Inoculation trials conducted in April and May, 1954, induced typical symptoms of yellow dwarf to develop in oats and barley 15 days after removal of aphids. The symptoms produced on oats and barley, the positive aphid transmissions, and failure of mechanical transmission indicate that this virus is identical or closely related to the barley yellow dwarf virus as first described in California. This is the first report of a virus disease of cereals in western Europe. The disease is unlikely to become economically important in Holland because aphids do not become abundant until the cereal crops are nearing maturity.

*Variation in pathogenicity of isolates of Armillaria mellea.* RAABE, ROBERT D. The pathogenicity of subcultures of different isolates of *Armillaria mellea* (Vahl) Quél. was tested by inoculating peach, pine, and dwarf dahlia seedlings in pots in the greenhouse. The subcultures used were originally from the following hosts and areas: peach (Ceres, Calif.), peach (Kingsburg, Calif.), 2 from red pine (Oconto, Wis.), white pine (Oconto, Wis.), western white pine (Noxon, Mont.), pear (Watsonville, Calif.), ceanothus (Marin County, Calif.), pelargonium (Oakland, Calif.), and juniper (Berkeley, Calif.). A subculture of *Clitocybe tabescens* (Scop.) Bres. (now considered by some as synonymous with *Armillaria mellea*) from peach (North Carolina) was also included. Inoculum was prepared by growing the subcultures on autoclaved pieces of pear branches that were nearly submerged in water. When the fungus was established, these blocks were buried in several inches of ground sycamore twigs in the bottoms of the pots, and the plants to be tested were then planted on top of this inoculum. Within a year, marked differences were found to exist in the pathogenicity of the isolates. Several were extremely pathogenic and killed all plants inoculated, others killed only some, and still others failed to kill any.

*Field studies of rose rootstock infections by the Verticillium wilt fungus.* RAABE, ROBERT D., AND STEPHEN WILHELM. Three years of field observations and tests have indicated that rose rootstocks commonly known as Ragged Robin, Odorata Multiflora (thorny, Burr and Grifferaie forms) are very susceptible to Verticillium wilt caused by *V. albo-atrum;* that Dr. Huey (Shafter) is less susceptible, and that Manetti is resistant. In tests with Red Delight and Red Garnet on these roots, inoculation by budding with a knife contaminated with Verticillium spores yielded the highest percentage of diseased plants and bud failures. Brushing a spore suspension on the lopping wound 1 month after budding yielded a lower percentage of infected plants. A low percentage of infected (conspicuously stunted) plants resulted when buds were taken from naturally infected wood. Eight days after budding, the budded area was flooded with irrigation water contaminated with Verticillium spores. No infections resulted. Once introduced into susceptible stocks, Verticillium grew readily through the wood, but growth through Manetti was insignificant. When inoculated by root dipping in a spore suspension and planting in infested soil, Manetti occasionally was invaded but overcame the disease and was pathogen-free after the first year. When budded with a contaminated knife, Manetti tended to restrict Verticillium to the tissues of the bud union, with the result that the top variety escaped invasion.

*Growth of tobacco mosaic virus in lesions produced on Nicotiana glutinosa.* RAPPAPORT, I., AND S. G. WILDMAN. Leaves of *Nicotiana glutinosa* were inoculated with TMV and kept under controlled light and temperature conditions. For 5 days after the first visible appearance of necrosis, the infectivity in a lesion, as compared to the infectivity of a standard purified virus preparation, was directly proportional to the lesion area. The lesion's radial growth was a linear function of time. From this rate of lesion spread, it was calculated that a perimeter of single cells became necrotic and was added on to the lesion in about 4 hours. Cytological examination of fresh leaf sections cut through the necrotic area and adjoining living tissue revealed degenerative changes in 3–4 cells beyond the sharply defined area of dead cells. Assuming that these changes reflected early virus activity in cells still alive, it was calculated that approximately 12 hours elapsed between infection and cell death. During this time (approximately) $2 \times 10^5$ virus particles were produced in each cell.

*The early time course of tobacco mosaic virus infection.* SIEGEL, A., AND S. G. WILDMAN. Evidence for the participation of no more than 1 virus particle in the establishment of a TMV lesion on *Nicotiana glutinosa* has been obtained by means of ultraviolet irradiation of the host-virus system. For 2 hours after inoculation, the UV survival curve of the infection centers is exponential and is the same as that of the *in vitro* virus. Furthermore, the *in vivo* curves are independent of the concentration of applied virus. If more than 1 particle participates in the establishment of a lesion, then multi-target curves would have been expected at high concentrations of applied virus. The initial events of infection have been followed by an extension of this type of experiment. At 4 to 5 hours after inoculation, the infection centers became more resistant to inactivation with UV although the exponential character of the curve suggests that multiplication of the virus has not as yet commenced. Seven

hours after inoculation, a multi-target curve is first obtained, indicating the presence of 3 particles per infection center. By 9 nours, 5 to 8 particles can be detected.

*A virus from gladiolus similar to common bean mosaic.* SNOW, GORDON F. A virus isolated from Chamouny variety gladiolus was 100 per cent seed-borne in *Phaseolus vulgaris* var. Bountiful in which the virus was first isolated. In subsequent transfers from Bountiful to Bountiful, the virus was only 40–50 per cent seed-borne. The host range is greater than that of common bean mosaic and includes alsike clover and subclover. It does not, however, include *Vicia faba* to which the usual yellow mosaic viruses from gladiolus are easily transferred. This unusual virus infects certain varieties of bean, such as Red Mexican U.I. #3 and Top Crop, that are resistant to typical common bean mosaic. It is exceptionally damaging to all the varieties of Blue Lake beans currently grown in Oregon. Attempts to transfer the virus mechanically to broadbean, red clover, white clover, white sweet clover, cucumber, snapdragon, tobacco, and zinnia were unsuccessful. The fact that the virus is seed-borne, its symptomatology, and its host range relate the virus to common bean mosaic although the 2 are not identical.

*Timing of treatments for control of snow mold of winter wheat.* SPRAGUE, RODERICK. Ceresan for Snow Mold (15.4 per cent *N*-(ethylmercuri-*p*-toluenesulfonanilide) is applied to emerged winter wheat after the Typhula basidiospores mature and before the winter snow comes. The first dates when mature basidiospores were seen in Washington are as follows: 1949, November 14; 1950, November 14; 1951, October 25; 1952, November 27; 1953, November 14; and 1954, about October 15. Except in 1954, spore discharge continued steadily from these dates until winter snows stopped their development. A few nearly mature basidiophores were seen in mid-October, 1954, but a prolonged rainy period plus development of black molds (Alternaria, Cladosporium) suppressed their further development. Control during these years was effective when Ceresan was applied from about November 1 to November 15, preferably during the first week in November. Control was less effective when delayed during open weather until December, even though snow mold was less severe during such years.

*Anomalous proteins associated with three strains of tobacco mosaic virus.* TAKAHASHI, WILLIAM N. Infection by 3 distinct strains of tobacco mosaic virus, severe, mild, and Holmes' rib grass, engenders the formation of anomalous proteins in tobacco plants. These proteins have been characterized by electrophoresis and have been found to be different and specific for each strain of virus. All were polymerized into rods resembling the virus.

*Evidence of retention of peach yellow bud mosaic virus in soil.* WAGNON, H. KEITH, AND JAMES R. BREECE. Following the planting in 1952 of healthy young nursery-grown peach trees in a virus test plot in California on land previously occupied by peach trees infected with peach yellow bud mosaic virus, 131 of 222 of the young trees showed the disease in 1953. In April, 1954, 5 cans of soil were collected from around the roots of 3 infected peach trees which had been in the plot since 1949. Two had shown yellow bud mosaic in 1952 and 1953 and the other only during 1953. Five additional cans for controls were filled with soil from an area where yellow bud mosaic is not known. Muir peach seeds were planted in all cans, and the resulting seedlings grew in the cans during 1954. In April, 1955, 9 of 22 seedlings growing in the cans of soil collected from around the roots of the infected peach trees exhibited leaf symptoms of yellow bud mosaic. The 23 seedlings that grew in the 5 control cans failed to show the disease.

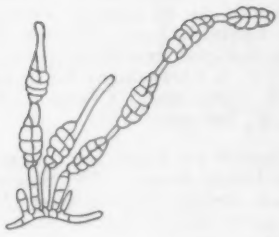
*Some factors affecting substrate colonization and growth of the Verticillium wilt fungus in soil.* WILHELM, STEPHEN. Under the conditions of the experiments performed, independent saprophytic growth of *Verticillium albo-atrum* could not be detected in 3 natural field soils during 2 months of winter. Verticillium grew vigorously in autoclaved soils, to a moderate degree in soils fumigated with chloropicrin (equivalent of approximately 400 lb. per acre), and to only a slight degree in soils fumigated with ethylene dibromide (equivalent of approximately 20 gal. Dowfume W-85 per acre). Three years of field tests with chrysanthemum as a test plant indicated that Verticillium slowly reinvaded chloropicrin-fumigated plots in which a high level of wilt control had been achieved. Primary colonization of previously sterilized tomato straw by *Chaetomium* sp. *Fusarium roseum, Gliocladium roseum,* and *Stysanus* sp. greatly impeded secondary colonization by Verticillium. Verticillium readily invaded tomato straw colonized by *Pythium ultimum,* another *Pythium* sp., *Trichoderma viride,* a Mucor, and a Cephalosporium. Growth of Verticillium in previously sterilized soil was stopped completely by *Gliocladium roseum.* The microsclerotia of Verticillium tended to resist Gliocladium.

*Freckle fruit of sweet cherry.* WILLIAMS, H. E. AND J. A. MILBRATH. During the summer of 1952, 2 Lambert sweet cherry trees near The Dalles, Oregon, developed a virus-like condition. At normal harvest time, most of the fruit on these trees was immature, undersized, and spotted with necrotic brown flecks. Severely affected fruits were deformed by shallow depressions. Mildly affected fruits were nearly smooth with most of the brown necrotic areas located near the stylar ends. No leaf symptoms were observed. One year after bud-inoculation, 2 15-year-old Lambert trees produced affected fruits on the inoculated branches and on some adjacent branches; the second year, the disease had spread over most of the trees. In 1953, 2 each of Bing, Lambert, and Napoleon cherry trees, 3 Improved Elberta peach trees, and 3 Tilton apricot trees were inoculated. Bing showed fruit symptoms in 1954 similar to those in Lambert but less severe. Affected fruits of Bing and Lambert trees matured about 2 weeks late and were small. Leaves on inoculated branches of both Bing and Napoleon were mottled. Napoleon developed no fruit symptoms. Peach and apricot trees developed no symptoms. The brown flecking has suggested the name "freckle fruit" for the disease.

*Generation time and the biological nature of viruses.* YARWOOD, C. E. The generation times (time for doubling of the population) under optimum conditions for 28 conventional organisms including bacteria, algae, yeasts, filamentous fungi, protozoa, insects, snails, oysters, birds, mammals, herbs, and trees have been shown to increase at a fairly regular rate with increase in body weight. The average generation time for these forms was about $4.5 \sqrt{10^{20.4}}$ X minutes when X was the body weight in grams. The published generation times of 2 bacteriophages were very close to this value. The generation times for 5 tissue invading viruses, including tobacco mosaic virus, tobacco necrosis virus, and southern bean mosaic virus were larger than expected from this formula. The generation times for free living bacteria and viruses (bacteriophages) were much shorter than for tissue invading bacteria and viruses. If the generation times for tissue invading viruses be corrected by a factor by which free living bacteria and viruses are known to differ from tissue invading forms, the generation time/body/weight relation for viruses will be approximately the same as that for conventional organisms.

DIFCO

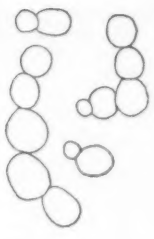
*Media for*



# MYCOLOGY

**DIAGNOSTIC**

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:



Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

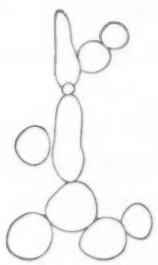
**CONTROL**

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

**CLASSIFICATION**

. . . and nutritional studies of fungi:



Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION,*
*including descriptions of these media and their use,*
*is available on request.*

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

**Antibiotics** in relation to crop usage was the subject discussed by several plant pathologists at the First International Conference on the Use of Antibiotics in Agriculture. The Conference was sponsored by the National Academy of Sciences—National Research Council and was held in Washington, D. C., October 19 to 21. Invitations to participate were extended to 44 leading scientists in Europe, South Africa, Australia, Latin America and the United States. Phytopathologists invited to participate included: E. C. Stakman; J. J. Christensen; W. J. Zaumeyer; J. G. Horsfall; C. M. Christensen; George L. McNew; H. C. Young; and J. C. Dunegan.

**New project leader** for sugar beet research work in the U. S. D. A. is Dewey Stewart. Mr. Stewart succeeds Dr. G. H. Coons who retired November 30. Mr. Stewart has been instrumental in the production of leaf spot resistant sugar beets and in breeding beets for resistance to curly top. He has also made contributions in the field of interspecific hybridization.

**Phytopathologist** E. C. Stakman has accepted an invitation from the University of California to give a series of public lectures under the Hitchcock Endowment. The lectures are scheduled for November and December.

**Graduate student** Robert J. Shepherd of Oklahoma A and M College was recently granted a Fulbright Scholarship for a year's study with the Virus Research Unit of the Agricultural Research Council, University of Cambridge, England.

**The deferment status** of scientists has been changed for the present although the Selective Service policy is not changed. Graduate students, teachers and men in essential research will be considered for deferment unless they are within six months of age 35. The procedure in securing deferment is through the Local Board as defined by law; however, when an appeal is made the Scientific Manpower Commission should be advised. At the time of filing the appeal with the Local Board a copy of the man's file and data justifying the man's deferment should be mailed to Dr. Howard A. Meyerhoff, Executive Director, Scientific Manpower Commission, 1530 P St., N. W., Washington 5, D. C. The information in this office will assist in directing the appeal through the State Offices for final consideration in the Selective Service Office. Dr. Meyerhoff reports: "Selective Service Headquarters has ruled favorably upon 95% of the cases that the Commission has placed before it."

**Extension plant pathologist** R. C. Rose of the University of Minnesota recently accepted a position as Plant Pathologist with the International Cooperative Administration in El Salvador, Central America. Mr. Rose was affiliated with the University of Minnesota for 35 years.

**Head** of the U. S. Market Pathology Laboratory in New York, New York, is the new title for phytopathologist, B. A. Friedman.

*Editor:* K. W. KREITLOW, Plant Industry Station, Beltsville, Md.


New fungicide treatment protects Potato Seed Pieces!

PHYGON-XL

controls rot up to 30 days

**PHYGON-XL** is one of the most economical and effective fungicides for destroying the fungi which attack unprotected seed potatoes in the ground. Phygon gives you better stands, higher yields, higher quality. Seed pieces won't rot up to 30 days after treatment, hence planting can be delayed in case of rain.

Phygon-XL® is today's cheapest, most effective organic fungicide for the control of fungus diseases on fruit trees, row crops and ornamentals.

Naugatuck Chemical

Division of United States Rubber Company

Naugatuck, Connecticut

US RUBBER

producers of seed protectants, fungicides, miticides, insecticides, growth retardants, herbicides: Spergon, Phygon, Aramite, Synklor, MH, Alanap, Duraset.

# INDEX FOR VOLUME 45

AUTHOR AND SUBJECT INDEX. New species and varieties in bold face type. Junior authorship indicated by pages in parentheses. Illustrations are included.


Aabulba, nematode control, 347
Aaventa, nematode control, 347
*Aceria* spp., see under Insects, mites
Achromycin, peach rosette virus, 348
Acids, organic, effect on TMV multiplication, 291
Acti-dione, see under Fungicides
Actinomycetes, *Pyrenochaeta terrestris*, antagonism, 440; **illuss. 441**
*Sclerotium rolfsii*, antagonism, 417; **illus. 418**
Actinomycin, soil, production and role, 407
ADAM, ANGELOS V., 285
ADAMS, ROBERT E., 464, 477
*Aerobacter* spp., *Sclerotium rolfsii* effect, **illus. 418**
*Agaricus compestris*, see Mushroom
*Agathis stigmaterus*, 37
Agrimycin, see under Antibiotics
*Agrobacterium rubi*, blackberry cane gall, 56, **illus. 57**
*tumefaciens*, antibiotics, inhibition, 618; **illuss. 621, 623**
ALCORN, STANLEY M., 272, 692
Aldrin, see under Insecticides
Alfalfa crown rot, HCN associated with, 667; **illuss. 668, 670, 672**
nematode, stem, control, 348
root-knot, varietal resistance, 70; **illus. 71**
Stemphylium leafspot, 352; **illus. 353**
tobacco anthracnose in, 465
white spot, genetic character, 289
ALLEN, O. N., (618)
ALLEN, PAUL J., 259
ALLINGTON, W. B., 595
ALLMENDINGER, D. F., (110)
Almond bud failure in peach, 323; **illuss. 325, 326**
*Alternaria* in cotton seedlings, control, 350
fungus culture, 466
*solani* in tomato fruit tumor, 136
sp. in apple, 392
spp., grape decay, 229
medium for, 461
tomato anthracnose, 340
*tomato*, temperature effect, 168; **illuss. 169, 171**
ALTMAN, JACK, 183
*Amaranthus retroflexus*, *Pyrenochaeta terrestris* in, 350
Amberlite 1R-120, antifermentative for culture of *Panagrellus redivivus*, 184
American Phytopathological Society: abstract, Southern Division, 463
Preliminary Symposia, Atlanta, Dec. '55, 442
reports and abstracts; Northeastern Division, 1954-55 winter meeting, 183
Pacific Division, 37th annual meeting, 692
Potomac Division, 1954-55, 464
Southern Division, 52nd annual meeting, 346
Amino acids, *Pseudomonas tabaci* toxin, modes of action, 659; **illuss. 660-663**
AMMANN, ALFRED, 559
*Amphorophora rubi*, strawberry vein banding virus, 307
ANDERSON, E. J., 238
ANDERSON, H. W., (285)
Antagonism, actinomycetes, *Pyrenochaeta terrestris*, 440; **illuss. 441**
*Sclerotium rolfsii*, 417; **illus. 418**
*Bacillus subtilis*, *Rhizoctonia* sp., 252; **illuss. 255, 257**
bacteria, *Sclerotium rolfsii*, 417; **illus. 418**
*Bacterium herbicola*, *Erwinia* spp. and *Corynebacterium flaccumfaciens*, 466
*Xanthomonas* and *Pseudomonas* spp., 466
*Chaetomium* sp., *Verticillium albo-atrum*, 696
fungi, *Sclerotium rolfsii*, 417; **illus. 418**
Antagonism—*continued*
*Fusarium roseum*, *Verticillium albo-atrum*, 696
*Gibberella zeae* to *Tilletia* spp., 288
*Gliocladium roseum*, *Verticillium albo-atrum*, 696
*Helminthosporium sativum* to *Tilletia* spp., 288
*Pyrenochaeta terrestris*, actinomycetes, 440; **illuss. 441**
*Rhizoctonia* sp., *Bacillus subtilis*, 252; **illuss. 255, 257**
*Sclerotium rolfsii* soil organisms, 417; **illus. 418**
*Stysanus* sp., *Verticillium albo-atrum*, 696
*Tilletia* spp., *Gibberella zeae*, 288
*Helminthosporium sativum*, 288
*Verticillium albo-atrum*, soil organisms, 696
Anthocyanosis of *Prunus* spp., X-disease infected and carbohydrate content of leaves, 184
Anthracnose, cucumber seed transmission, 348
lettuce, etiology, host range, 375; **illuss. 377, 379**
tobacco, host range, 465
tomato foliage, inoculum source, 338; **illuss. 340, 342-344**
Antibiosis, *Candida intermedia*, *Physalospora tucumanensis*, 37; **illus. 38**
*Pyrenochaeta terrestris*, actinomycetes, 440; **illuss. 441**
Antibiotics, general, *Agrobacterium tumefaciens* and gall tissue, 618; **illuss. 621, 623, 624**
soil, production and role, 407
Antibiotics, achromycin (tetracycline complex), peach rosette virus, 348
actinomycin, soil, production and role, 407
agrimycin (1 part terramycin to 10 streptomycin), peach rosette virus, 348
pepper bacterial spot, 465
philodendron leaf spot, 694
antimycin A., *Venturia inaequalis* resistance to, 467; **illus. 469**
aureomycin, *Agrobacterium tumefaciens* and gall tissue, 618
hydrochloride (chlortetracycline), peach rosette virus, 348
pear blast, 629
candicidin A, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
chloramphenicol, *Agrobacterium tumefaciens* and gall tissue, 618; **illuss. 621, 623, 624**
chlorotetracycline and amphomycin, Dutch elm disease, 185
circulin, soil, production and role, 407
clavacin, *Agrobacterium tumefaciens*, and gall tissue, 618; **illus. 623**
cycline compounds, peach rosette virus, 348
cycloheximide, mold control in *Graphium ulmi* isolation, 185
*Verticillium* sp. in elm, 185
endomycin, turf brown patch, 186
filipin from *Streptomyces* **filipinensis** nov. sp., 559; **illus. 560**
neomycin, soil, production and role, 407
noformicin from *Norcardia formica*, antiviral effect, 281
patulin, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
penicillin, *Agrobacterium tumefaciens* and gall tissue, 618; **illus. 623**
pleocidin, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
polymyxin, *Agrobacterium tumefaciens*, and gall tissue, 618; **illuss. 621, 623**
B sulfate, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
streptomycin, *Agrobacterium tumefaciens* and gall tissue, 618; **illus. 623**
nitrate, pepper bacterial leaf spot, 183
pear blast, 629

Antibiotics, streptomycin—*continued*
*Pyracantha*, translocation, 692
sulfate, bacterial soft rot, spinach, 88
streptothricin hydrochloride, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
subtilin, soil, production and role, 407
terramycin, *Agrobacterium tumefaciens* and gall tissue, 618; **illuss. 621, 623, 624**
peach rosette virus, 348
tetracycline, *Pyracantha*, translocation, 692
thiolutin, Dutch elm disease, 185
*Verticillium* sp. in elm, 185
tyrothricin, *Agrobacterium tumefaciens* and gall tissue, 618
viomycin, soil, production and role, 407
Antimycin A, *Venturia inaequalis*, resistance to, 467; **illus. 469**
*Aphis* spp., see under Insects, aphid
Apple black rot control, 392; **illuss. 393, 395**
black rot, light effect on pycnidia of pathogen, 22; **illus. 23**
foliage injury, glyodin and arsenic, 185
fruit rot, control, 466
green mottle, 186
leafspot control, 392; **illuss. 393, 395**
light effect on pycnidia of pathogen, 22; **illus. 23**
mosaic virus in peach, 292; **illus. 293**
"pout," *Xiphinema* sp., control, 464, 477
*Xiphinema* sp. in, control, 464
Apple, J. Lawrence, 346
Arasan, see also Fungicides, thiram, *Rhizoctonia solani*, 466
Aristeo, Acosta C., 503
Ark, Peter A., (692)
*Armillaria mellea* pathogenicity of isolates, 695
Armstrong, G. M., (346)
Armstrong, J. K., 346
Arny, D. C., 518
Asparagus Penicillium crown rot, symptoms and control, 527; **illus. 528**
*Aspergillus*, fungus culture, 466
Aster, carnation ringspot virus, 464
celery-yellows virus, leafhopper transmission tests, 185
Fusarium wilt, sumac relationships, 346
Aureomycin, see under Antibiotics
Avocado Verticillium wilt, susceptibility, 635

*Bacillus cereus*, antibiotics effect, 407
*subtilis*, antibiotics effect, 407
*Rhizoctonia* sp., antagonistic, 252; **illuss. 255, 257**
Bacteria, *Sclerotium rolfsii*, antagonism, 417; **illus. 418**
Bacterial blights, bean, resistance testing method, 519; **illus. 520**
canker, tomato, anatomy, 267; **illuss. 268-270**
diseases, soft rot, spinach, control in packaged, 88
leaf spot, pepper, control, 183
spot, pepper, control, 465
Bacteriophage, 196
*Xanthomonas malvacearum*, attacking, 49, **illus. 50**
*pruni*, production, 580
spp. strains, specificity, 596; **illus. 597**
*Bacterium herbicola, Corynebacterium flaccumfaciens*, antagonism, 466
*herbicola, Erwinia* spp., antagonism, 466
*Pseudomonas* spp., antagonism, 466
*Xanthomonas* spp., antagonism, 466
Bain, Douglas C., 35, 55
Baker, Kenneth F., (509)
Bald, J. G., 156, 693
Barley, calcium sulfamate effect, 503
germination, water-soak seed treatment, effect, 518
loose smut, longevity of pathogen in seed, 637
varietal resistance, 73; **illus. 77**
water-soak seed treatment, control, 518

Barley—*continued*
net blotch, inheritance of resistance, 174
Pythium root rot, adaptation, 97; **illuss. 98, 100**
scald control, 350
stem rust control, 503
wheat streak mosaic virus, mite spread, 116
yellow dwarf virus, Holland, 695
Baxter, Dow V., (694)
Bean bacterial blights resistance, testing method, 519; **illus. 520**
carnation ringspot virus, 464
mosaic virus, a gladiolus—similar to, 696
powdery mildew, chemicals in leaves, 43, **illus. 45**
rust, chemicals in leaves, 43, **illuss. 45, 46**
southern mosaic virus, noformicin effect, 281
seed maturation effect, 17
thiouracil effect, 281
Beet, Fusarium wilt, spinach, relationships, 346
sugar, tobacco anthracnose in, 465
yellow virus, mechanical transmision, 233; **illus. 234**
Begonia, tobacco anthracnose in, 465
*Bellis perennis*, see English Daisy
Bennett, C. W., (233), 531
Benzene hexachloride, see under Nematocides
Bernstein, L. B., 580, (596)
Bever, Wayne M., (513)
Biographical sketch, Heald, Frederick Deforest, 409, **illus.**
Manns, Thomas Franklin, 241; **illus.**
Miles, Gilbert Frazer, 1, **illus.**
Muncie, Jesse Howard, 523, **illus.**
Tehon, Leo Roy, 115, **illus.**
Tucker, Clarence Mitchell, 351, **illus.**
Biotin, *Sclerotinia trifoliorum*, 39
Bis(dimethylthiocarbamoyl) disulfide, see Fungicides, thiram
Bjornsson, Ida, (465)
Black, L. M., 208
Blackberry cane gall, 56; **illus. 57**
mosaic virus complex, 272; **illus. 273**
Blueberry stunt virus, wild sources, 465
Bluegrass stem rust control, 186
Bloom, J. R., 443
Book listing, 90, 408, 463, 638
Boone, D. M., (467)
Boosalis, Michael G., (293)
Boothroyd, C. W., (186)
Bordeaux, spruce canker, 686
Boron, see also under Nutrition of microorganisms
pear blossom blight, twig dieback, deficiency, control, 110; **illuss. 111, 113**
Borthwick, H. A., (465)
*Botryodiplodia phaseoli*, potato charcoal rot, India, 91
*Botryosphaeria ribis*, apple fruit rot phase, control, 466
medium for, 461
*Botrytis cinerea*, grape decay, 229
*cinerea* in grapes in storage, control, 137
in tomato fruit tumor, **illus. 133**
*gladiolorum* nutrition tests, 676; **illus. 677**
Bourne, B. A., 37
Boxwood, *Helicotylenchus*, pathological effect, 464
Brandes, G. A., (350)
Braun, Alvin J., 183
Braun, Armin C., 659
Breece, James R., (696)
Bretz, T. W., 351
Brierley, Philip, 2, 464
Brock, Thomas D., (559)
Browning, J. A., (490)
Bruehl, G. W., 97

Cabbage blackrot, 55
blackrot inheritance of resistance, 35; **illus. 36**
Cactus Helminthosporium stem rot, symptoms and control, 509; **illuss. 510, 511**

Cadmium succinate, snow mold, 59; **illus. 61**
Cairns, Eldon J., 346
Calcium, see also under Nutrition
  sulfamate, see under Fungicides
*Callistephus chinensis*, see China-aster
*Calonyction aculeatum* for sweet potato internal cork assay, 185
Campbell, Colin E., 644
Campbell, Robert N., 485
Candicidin, see under Antibiotics
*Candida*, fungus culture, 466
  *intermedia*, *Physalospora tucumanensis*, antibiosis, 37; **illus. 38**
*Capitophorus fragaefolii*, strawberry vein banding virus, 307
*Capsicum frutescens*, see Pepper
Captan, see under Fungicides
Carbon dioxide effect on *Rhizoctonia solani* distribution in host, 693
Carnation mosaic virus, 464
  ringspot virus, 464
Caroselli, Nestor E., 183, 184
Carpenter, T. R., 520
Carter, Herbert E., (559)
Carter, J. C., 115
*Cattleya* Fusarium wilt, 599; **illuss. 600, 601**
  leaf necrosis, Cymbidium mosaic, 327; **illuss. 328, 329**
Celery, peach yellow leaf roll virus host, 694
*Cephalocereus* spp., (see also Cactus) top rot, **illus. 510**
*Cephalosporium gregatum*, asexual reproduction, 293; **illus. 294**
  spp., lettuce root rot, associated with, 184
*Cercospora capsici*, pepper resistance, 347
Ceresan, see under Fungicides
*Chaetomium* sp., *Verticillium albo-atrum*, antagonistic, 696
Chandler, Philip A., (693)
Chandler, W. A., (185)
Charcoal rot, potato, India, 91
Chard, cucumber mosaic virus, aphid-vector studies, 217
  Fusarium wilt, spinach, beet, relationships, 346
Chemical names, fungicides, active ingredients, 295
Chemotherapeutants, 8-hydroxyquinoline benzoate, turf brown patch, 186
  8-hydroxyquinoline sulphate, turf brown patch, 186
Chemotherapy, peach rosette virus, antibiotics, 348
Chenopodiaceae, Fusarium wilt, 346
Cheo, Pen Ching, 17, 184, (350)
Cherry buckskin virus, leafhopper vectors, 694
  "freckle fruit" virus, 696
  necrotic ring spot virus, *Prunus tomentosa* index plant, 320; **illuss. 321-323**
  Montmorency, 565
Chessin, M., 288
China-aster, *Stemphylium* **floridanum** in, 11
Chloramphenicol, *Agrobacterium tumefaciens* and gall tissue, 618; **illuss. 621, 623, 624**
Chloranil, see under Fungicides
*Chlorella vulgaris*, *Pseudomonas tabaci* toxin and methionine in, **illus. 660**
Chloride, pineapple injury and control, 590; **illuss. 591, 592**
*Chlorogenus callistephi* var. *californicus*, leafhopper transmission tests, 185
3-*p*-Chlorophenyl-5-methylrhodanine, see Nematocides, N-244
Chlorophyll role in obligate parasitism, 186
Chlorotetracycline and amphomycin, Dutch elm disease, 185
Chokecherry X-disease, anthocyanosis and carbohydrate content of leaves, 184
Christensen, J. J., (639)
Chrysanthemum aspermy virus, 2; **illus. 5**
  flower distortion, 2; **illus. 5**
  mosaic virus, 2; **illus. 5**
  rust distribution, 644
    temperature, 644; **illuss. 645-647**
  viruses, 2; **illus. 5**
Citric acid effect on TMV multiplication, 291
Citrus inoculation method, 517, **illuss.**
  mulch-sprinkler, nematophagus fungi control, 693
  nematophagus fungi, 693
  tristeza, yellows and pitting complex, 222
  water mold parasites, swarming, 694
*Cladosporium cucumerinum*, resistant varieties, 451
  *herbarum*, grape decay, 229
Clavacin, *Agrobacterium tumefaciens* and gall tissue, 618; **illus. 623**
Clayton, E. E., (349), (463)
*Clitocybe tabescens* pathogenicity of isolates, 695
Clover, *Sclerotinia trifoliorum*, toxin, physiological studies, 39
  seed, pathogen isolation, method, 94; **illuss. 95, 96**
  tobacco anthracnose in, 465
Code, biological taxonomic, 58
Cole, Herbert, Jr., (405)
Coleman, N. T., (348)
Coleus, tobacco anthracnose in, 465
*Colletotrichum*, fungus culture, 466
  *lagenarium*, cucumber seed transmission, 348
  *phomoides*, *medium* for, 461
    tomato foliage, inoculum source, 338; **illuss. 340, 342-344**
  sp. host range, 465
*Collodonus geminatus*, see under Insects, leafhopper
Conifer canker, *Valsa kunzei* relation to, 686; **illus. 688**
Copper, see under Fungicides
Corbett, M. K., 148
Corn, albino chlorophyll relationship in parasitism studies, 186
  crown rot, *Helminthosporium turcicum* incitant, 291; **illus. 292**
  downy mildew, histology, 350
  golden nematode, stimulation of larval emigration, 184
  *Pyrenochaeta terrestris* in, 350
  root-knot nematode, **illus. 543**
  sugar cane virus, electron microscopy, 694
  wheat streak mosaic virus, mite spread, 116
*Corynbacterium flaccumfaciens*, *Bacterium herbicola*, antagonism, 466
  *michiganense*, medium for, 461
    tomato, anatomy of infected plants, 267; **illuss. 268-270**
Costa, A. S., 233
Cotton blight, bacteriophage attacking pathogen, 49, **illus. 50**
  Fusarium wilt, nematode studies, 349
  *Helicotylenchus* sp. in, 349
  *Hoplolaimus coronatus*, 349
  *Meloidogyne* sp. in, 349
  nematode, Fusarium wilt studies, 349
  nub-root, seedling disease expression, 413; **illus. 415**
  *Pratylenchus* sp. in, 349
  root-knot nematode, 540
  seedling disease control, 350
Couch, Houston B., 63, 375
Coumarin effect on spore germination, *Puccinia graminis*, 259; **illus. 265**
Cowpea, cucumber mosaic virus, resistance, 563
  Fusarium wilt, resistance of Brown Sugar Crowder, 347
Cox, R. S., 291
Crag, see under Fungicides
*Criconemoides* sp., pathogenicity in tobacco and Spanish peanuts, 347
Crittenden, H. W., 347
Crossan, D. F., (465)
Crown gall, tomato, marigold, antibiotics, inhibition, 618; **illuss. 621, 623, 624**
Cucumber anthracnose, seed transmission, 348
  carnation ringspot virus, 464
  Fusarium wilt, 435; **illuss. 436, 437**
  mosaic virus, cowpea resistance, 563

Cucumber mosaic—*continued*
resistant varieties, 451
southern, insect transmision, 217; **illus. 218**
scab resistant varieties, 451
tobacco anthracnose in, 465
ring spot virus, nutrition, 567; **illus. 570**
Curculin, soil, production and role, 407
CURL, E. A., (581)
*Curvularia lunata*, nutrition tests, 676; **illus. 677**
*Cuscuta subinclusa*, see Dodder
Cyano (methylmercuri) guanidine, see Fungicides, Panogen
Cycloheximide, see under Antibiotics
and ferrous sulphate, see Fungicides, Acti-dione-Errated
Cymbidium mosaic virus hosts, electron microscopy, 327; **illuss. 328, 329, 331**
*Cytospora kunzei* var. **kunzei** var. nov., 689
var. **piceae** var. nov., 689

Dahlia spotted wilt, elimination, 224; **illus. 225**
Daisy, English, *Marssonina panattoniana* in, 375
DARROW, GEO. M., 464
*Datura stramonium*, Cymbidium mosaic virus in, 327; **illus. 331**
*stramonium*, potato virus X in, 313
yellow-net virus, 479, **illus.**
DAVIS, B. H., (183)
DAVIS, LILY H., (509)
D-D, see under Nematocides
Depolymerase, see under Enzymes
DESJARDINS, PAUL R., (334)
DEWOLFE, T. A., 693, (694)
DEZEEUW, D. J., 523
*Dianthus barbatus*, aphid transmission of carnation mosaic virus, 464
*Diatraea saccharalis*, yeast associated with, antibiosis, sugar cane rot, 37
1,2,-Dibromo-3-chloropropane, see under Nematocides
1,2-Dibromoethane, see Nematocides and Soil Fumigants, Dowfume W-85
Phillips hydrocarbon mixture, see Nematocides, PN-20
Dichlone, see under Fungicides
2,4-Dichloro-6 (*o*-chloroanilino)-*syn*-triazine, grape decay in storage, 137
2,3-Dichloro-1, 4-napthoquinone, see Fungicides, dichlone
Dichloropropene-dichloropropane, see Fungicides, DD and Soil Fumigants, DD
DICKSON, J. G., (667)
DIENER, URBAN L., 141, 654
*Digitaria sanguinalis*, *Pyrenochaeta terrestris*, 350
3-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide, see Fungicides, Acti-dione
DIMOCK, A. W., (644)
DIMOND, A. E., (79)
Dinitrophenol, effect on spore germination, *Puccinia graminis*, 259; **illus. 265**
tomato Fusarium resistance, effect, 303
*Diplocarpon rosae* control, 465
Disease resistance, see Resistance
Disodium ethylenebis [dithiocarbamate], see Fungicides, nabam
Dithane Z-78, see Fungicides, zineb
*Ditylenchus* spp., see under Nematodes
Dodder, blueberry stunt virus, 465
curly top virus, water pimpernel, transmission, 531; **illuss. 532, 535**
strawberry vein banding transmission, 307
Dowfume W-85, see under Nematocides
Downy mildew, corn, 350
sorghum, India, 128; **illus. 130**
DROLSOM, P. N., (349)
DROPKIN, V. H., (26)
DUNLEAVY, JOHN, 252
DURBIN, RICHARD D., 509, 693

*Echinochloa colona*, *Pyrenochaeta terrestris* in, 350
Eggplant, *Pseudomonas solanacearum*, pathogenicity of isolates, 348
EISENSTARK, A., (580), 596
Electron microscopy, apparatus for frozen-dried specimens, **illus. 190**
Cymbidium mosaic identity, 331
methods, 187; **illuss. 189, 190, 192, 194**
sugar cane mosaic virus, 694
TMV, 603; **illuss. 604-606**
tomato ring spot virus, 334; **illus. 336**
wheat mosaic virus, 348
Electrophoresis, squash mosaic virus, 145
tomato ring spot virus, 334; **illus. 335**
virus assay techniques, 211
*Eleusine indica*, *Pyrenochaeta terrestris* in, 350
ELLIOTT, EDWARD S., (673)
Elm, Dutch elm, disease, antibiotic tests, 185
EMTS, bluegrass stem rust, 186
*Endoconidiophora fagacearum* longevity under mineral oil, 405
mat formation studies, 485; **illuss. 487, 488**
transmission by squirrels, 581; **illus. 582**
Endomycin, turf brown patch, 186
*Endothia parasitica*, light, response to, 465
ENGLISH, HARLEY, (239)
*Entomosporium macalatum*, hawthorn leaf spot, 55; **illus. 55**
Enzymes, depolymerase, *Fusarium oxysporum* f. *lycopersici*, 381, 524; **illus. 525**
methylesterase in *Fusarium oxysporum* f. *lycopersici*, 79; **illus. 82**
pectic, *Fusarium oxysporum* f. *lycopersici*, 381, 524; **illus. 525**
pectin methylesterase, *Fusarium oxysporum* f. *lycopersici*, 381, 524; **illus. 525**
polygalacturonase in *Fusarium oxysporum* f. *lycopersici*, 79; **illus, 82,** 534; **illus. 525**
protopectinase, *Sclerotinia trifoliorum*, 39
*Erwinia amylovora*, medium for, 461
*atroseptica*, potato suberin and periderm formation, 649; **illuss. 651-653**
spp., *Bacterium herbicola*, antagonism, 466
*Erysiphe polygoni*, chemicals in infected leaves, 43; **illuss. 45, 46**
Ethionine, *Pseudomonas tabaci* toxin, modes of action. **illus. 662**
*N*-(Ethylmercuri-*p*-toluenesulfonanilide), see Fungicides, EMTS
Ethylmercury phosphate, Fungicides, Ceresan, New Improved
EVERETT, H. L., (186)

*Fabraea maculata*, imperfect stage in India-hawthorn, 55; **illus. 55**
FEDER, WILLIAM A., (347)
FELDMESSER, JULIUS, 347
Ferbam, see under Fungicides
FERGUS, CHARLES L., 405
FERGUSON, JOHN, 693
Fermate, see Fungicides, ferbam
Ferric dimethyldithiocarbamate, see Fungicides, ferbam
FERRIS, J. M., 184
FERRIS, VIRGINIA ROGERS, 546
Fig mosaic virus mite transmission, 52; **illuss. 53, 54**
Filipin from *Streptomyces* **filipinensis** nov. sp., 559; **illus. 560**
FINK, HARRY C., 320
FISCHER, GEORGE W., 409
Flax, nub-root, seedling disease expression, 413; **illus. 415**
rust, host-parasite interaction, genetics, 680
FLOCK, R. A., 52
FLOR, H. H., 680
Formaldehyde, lily bulbs, 693
FOSTER, H. H., 347

FOSTER, VIRGINIA, 599
*Fragaria chiloensis, Verticillium albo-atrum*, resistance, 387
spp., variegation, 464
*vesca*, indexing strawberry virus, 183
*virginiana, Verticillium albo-atrum*, resistance, 387
FRAZIER, NORMAN W., 307
FREEMAN, T. E., 440
FRENCH, DAVID W., (485)
FREY, K. J., 490
FRIEDMAN, B. A., 184
FULKERSON, JOHN F., 22
Fungi, filipin from *Streptomyces* **filipinensis**, nov. sp., effective against, 559; **illus. 560**
light, response to, 465
nematophagus, in citrus, 693
*Sclerotium rolfsii*, antagonism, 417; **illus. 418**
Fungicides, general, asparagus Penicillium crown rot, 529
chemical names, 295
fungi mutants, resistance, *Venturia inaequalis*, 467; **illus. 469**
*Rhizoctonia solani* soil factors effect, 466
rose blackspot, insecticide and miticide combinations, 465
soil testing, *Phytophthora cinnamomi*, 398; **illus. 401**
Fungicides, Acti-dione (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide), *Phytophthora cinnamomi*, 398; **illus. 401**
Acti-dione, *Rhizoctonia solani*, 466
Errated (cycloheximide and ferrous sulphate), bluegrass stem rust, 186
Arasan, see also Fungicides, thiram, *Rhizoctonia solani*, 466
B-622(2,4-dichloro-6-(*o*-chloroanilino)-*syn*-triazine) grape decay in storage, 137
bis(dimethylthiocarbamoyl) disulfide, see Fungicides, thiram
Bordeaux, spruce canker, 686
cadmium succinate, snow mold, 59; **illus. 61**
calcium sulfamate, barley, effect, 503
bluegrass stem rust, 186
oats, effect, 503
stem rust, 503
wheat, effect, 503
fungicides, captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) coined name, 100
apple black rot and leaf spot, 392
fruit rot, 466
cactus Helminthosporium stem rot, 509; **illus. 511**
grape decay in storage, 137
peach brown rot, 347
scab, 347
storage rot, 347
rose blackspot, 465
carbamate spray, philodendron leaf spot, 694
ceresan, wheat snow mold control, 696
M, *Septoria avenae* in oats, 182; **illus. 182**
New Improved, (ethylmercury phosphate), *Phytophtora cinnamoni*, 398; **illus. 401**
chloranil (tetrachloro-*p*-benzoquinone) coined name, 100
turf snow mold, 59
Copper Compound A (basic copper chloride), tomato damping-off, 347
oxysulfate, rose blackspot, 465
spray, philodendron leaf spot, 694
Crag 5400 (α,α-trithiobis(*N*-dimethylthioformamide)), grape decay in storage, 137
Potato Fungicide (copper zinc chromate, $2.5CuO \cdot 0.6CdO \cdot ZnO \cdot 10CaO \cdot CrO_3$), tomato damping-off, 347
cycloheximide, grape powdery mildew, 695
peach brown rot, 695
2,3-dichloro-1, 4-naphthoquinone, see Fungicides, dichlone 100
Fungicides—*continued*
dichlone (2,3-dichloro-1,4-naphthoquinone) coined name, bluegrass stem rust, 186
turf brown patch, 186
disodium ethylenebis [dithiocarbamate], see Fungicides, nabam
Dithane Z-78, see also Fungicides, zineb, *Rhizoctonia solani*, 466
EMTS (*N*-(ethylmercuri-*p*-toluenesulfonanilide)), bluegrass stem rust, 186
ferbam (ferric dimethyldithiocarbamate) coined name, 100
cactus Helminthosporium stem rot, 509; **illus. 511**
lily bulbs, 693
Lysol, lily Rhizoctonia rot, 156
*Rhizoctonia solani*, 466
rose blackspot, 465
tomato damping-off, 347
ferric dimethyldithiocarbamate, see Fungicides, ferbam
sulfate, glyodin and arsenic, apple foliage injury preventative, 185
formaldehyde, lily bulbs, 693
glyodin (2-heptadecyl-2-imidazoline acetate) coined name, 100
and arsenic, apple foliage injury, 185
2-heptadecyl-2-imidazoline acetate, see Fungicides, glyodin
arsenate, apple foliage injury, 185
Karathane, rose blackspot, 465
Lysol-ferbam dip, lily Rhizoctonia rot, 156
maleic hydrazide, turf brown patch, 186
30, oats crown rust, 410
maneb (manganous ethylenebis [dithiocarbamate]) coined name, 100
bluegrass stem rust, 186
manganous ethylenebis [dithiocarbamate], see Fungicides, maneb
mercury chloride, snow mold, 59; **illus. 61**
turf brown patch, 186
methyl bromide (bromomethane) gladiolus, *Sclerotium rolfsii*, control and injury, 520
nabam (disodium ethylenebis [dithiocarbamate]) coined name, 100
Panogen(cyano(methylmercuri)guanidine), barley scald, 350
phenyl mercury, snow mold, 59; **illus. 61**
PMAS (phenyl mercury acetate), turf snow mold, 59; **illus. 61**
Puratized Agricultural Spray 6% (tris(2-hydroxyethyl)(phenylmercuri) ammonium lactate), lily bulbs, 693
*Rhizoctonia solani*, 466
C12-2525 (anilinocadmium lactate; phenylmercury derivatives of 2-mercaptobenzothiazole), tomato damping-off, 347
Semesan (30% 2-chloro-4-(hydroxymercuri) phenol), *Rhizoctonia solani*, 466
sodium sulfanilate, barley, 503
oats, 503
stem rust, 503
wheat, 503
Stauffer N-521 (tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione), *Phytophtora cinnamomi*, 398; **illus. 401**
N-869(sodium methyldithiocarbamate) pea root rot, *Fusarium solani*, 185
*Phytophthora cinnamomi*, 398; **illus. 401**
sulfur, rose blackspot, 465
wettable, peach brown rot, scab, storage rot, 347
tetrachloronitrobenzene, grape injury in storage, 137
tetrachloro-*p*-benzoquinone, see Fungicides, chloranil
tetramethylthiuram disulfide, see Fungicides, thiram
thiram (bis(dimethylthiocarbamoyl) disulfide) coined name, 100
snow mold, 59; **illus. 61**

**Fungicides, thiram**—*continued*
tetramethylthiuram disulfide, alternate name, 100
turf brown patch, 186
*N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide, see Fungicides, captan
Vancide 51, *Rhizoctonia solani*, 466
ZW (zinc dimethyldithiocarbamate; zinc 2-mercaptobenzothiazole), tomato damping-off, 347
zinc dimethyldithiocarbamate, see Fungicides, ziram
ethylenebis[dithiocarbamate], see Fungicides, zineb
zineb (zinc ethylenebis[dithiocarbamate]) coined name, 100
apple fruit rot, 466
bluegrass stem rust, 186
cotton seedling diseases, 350
rose blackspot, 465
tomato damping-off, 347
ziram (zinc dimethyldithiocarbamate) coined name, 100
*Rhizoctonia solani*, 466
tomato damping-off, 347
**Fungus culture conservation**, method comparison, 466
***Fusarium*** in cotton seedlings, control, 350
fungus culture, 466
*lini*, cultural characteristics, variation, 240
*moniliforme*, sugar cane red rot, 37
*nivale* in turf, control, 59; **illus. 61**
*oxysporum* f. *gladioli*, control, 693
nutrition tests, 676; **illus. 677**
f. *lycopersici*, enzyme role, 381
nutrient sprays effect on host response, 443
pectic enzymes, 79; **illus. 82**; 524; **illus. 525**
tomato resistance, 303
var. *nicotianae*, composition of leaves, 506
nutrition and metabolism, 350
rubrofusarin production, 350
tobacco resistance, 348
xanthone production, 350
f. *vasinfectum*, nematodes related to, in cotton, 349
*roseum*, *Verticillium albo-atrum* antagonistic, 696
*solani*, pea root rot control, 185
sp. in lily, 693
spp., cucumber, 435; **illuss. 436, 437**
lettuce root rot, associated with, 184
muskmelon, 435; **illus. 436**
nub-root, cotton and flax, 413
watermelon, 435; **illus. 436**
wilt, aster and sumac relationships, 346
beet and spinach, host relationships, 346
*Cattleya*, 599; **illuss. 600, 601**
cowpea, resistance of Brown Sugar Crowder, 347
spinach and beet, host relationships, 346
sumac and aster relationships, 346

**Galacturonic acid**, polygalacturonase produced, tomato Fusarium wilt, 79
**Gallegly, M. E.**, 103, 464, (473)
**Gammon, Earle T.**, (520)
**Garlic**, *Pyrenochaeta terrestris* in, 350
**Genetics**, alfalfa white spot, 289
barley resistance to net blotch, 174
cabbage blackrot resistance, 35; **illus. 36**
cowpea, cucumber mosaic virus, resistance, 563
flax rust, host-parasite interaction, 680
tomato resistance to *Phytophthora infestans* races, 103
*Ustilago kolleri*, haplo-lethal deficiency, 521, **illus.**
***Geranium maculatum*** variegated, chlorophyll role, studies with *Puccinia polygoni-amphibii*, 186
spp. bacterial stem and leaf disease, 613; **illus. 616.**
tobacco antharcnose ni, 465
***Gibberella zeae***, *Tilletia* spp., antagonism, 288
**Gilmer, R. M.**, 184
**Gilpatrick, J. D.**, (576)
**Gladiolus**, fungal pathogens, nutrients effect on growth and pathogenicity, 676; **illus. 677**

**Gladiolus**—*continued*
Fusarium disease, control, 693
hot-water treatment, 693
*Sclerotium rolfsii*, methyl bromide, control and injury, 520
soil treatment, 693
*Stemphylium* **floridanum** in, 15
a virus similar to bean mosaic, 696
*Gliocladium roseum*, *Verticillium albo-atrum*, antagonism, 696
*Glomerella cingulata*, marker strain, tomato anthracnose studies, 338
spp., medium for, 461
**Glyodin**, see under Fungicides
**Goheen, A. C.**, (465)
**Gold, A. H.**, (327), 693, 694
**Golden, A. Morgan**, 464
**Gomphrena**, carnation ringspot virus, 464
*globosa*, lettuce mosaic, 63
potato virus X in, 313
**Gothoskar, S. S.**, 303, 381, (524)
**Gottlieb, David**, (407), (559)
**Graham, J. H.**, 227, 633
**Graham, T. W.**, 347
**Grape** decay in storage, control, 137
powdery mildew control, 695
storage, decay forecasting, 229; **illuss. 230, 231**
**Grapefruit**, tristeza, yellows and pitting complex, 222
*Graphium ulmi*, antibiotic tests, 185
**Grass**, bent, nematode control, 350
brown patch control, 186
orchard, *Pleospora phaeocomes*, 633, **illus.**
tobacco anthracnose in, 465
wheat streak mosaic virus, mite spread, 116
**Grasso, Vincenzo**, 521
**Gray, Reed A.**, 281
**Green mottle** of apple, 186
**Griffith, R. B.**, 428
**Grogan, R. G.**, (267), (375)

**Hagedorn, D. J.**, (603), (665)
**Halma, F. F.**, (635)
**Hamilton, Richard I.**, 293
**Hannon, Chancellor, I.**, 11, 184
**Hansen, H. N.**, (55)
**Hansing, E. D.**, (8)
**Hare, W. W.**, 347
**Harrison, A. L.**, 347
**Harvey, John M.**, 137, 229
**Hashimoto, S.**, (693)
**Hawthorn**, Entomosporium leaf spot, 55; **illus. 55**
**Heald, Frederick Deforest**, biographical sketch, 409, **illus.**
**Herbert, T. T.**, 348
**Heggestad, H. E.**, 348, 463
**Heidrick, L. E.**, 250
**Held, Victor M.**, 39
*Helicotylenchus* sp., see under Nematodes
*Helminthosporium cactivorum*, symptoms and control, 509; **illuss. 510, 511**
*dictyoides* var. **phlei**, var. nov., 227; **illus. 227**
fungus culture, 466
*sativum*, oats resistance to, 219; **illus. 220**
*Tilletia* spp., antagonism, 288
*turcicum*, corn crown rot incitant, 291; **illus. 292**
medium for, 461
*victoriae* toxin production, 453; **illuss. 456, 457**
**Helms, Katie**, (493), 567
**Henderson, R. G.**, 348
***Hendersonula toruloidea*** in walnut, sunburn effect, control, 607; **illuss. 608, 609, 611**
**Heptachlor**, see under Nematocides
1,4,5,6,7,8,8,Heptachloro-3a, 4,7,7a-tetrahydro-4, 7-methanoindene, see Nematocides, Heptachlor
2-Heptadecyl-2-imidazoline acetate, see Fungicides, glyodin

Heterocaryosis, *Puccinia graminis* var. *tritici*, basis for variation, 639; **illuss. 641, 643**
*Heterodera* spp., see under Nematode
Heuberger, J. W., 241, (466)
Hewitt, Wm. B., (267)
Heyne, E. G., 8
Himelick, E. B., 581
Hogsett, J. N., (185)
Holmes, Francis O., 185, 224
Holmes, F. W., 185
Hooker, W. J., (313), (445), (523)
*Hoplolaimus coronatus* in cotton, 349
Horn, N. L., 348
Huffman, M. D., 278
Hutchinson, M. T., 465
Hwang, Shuh-Wei, (466)
Hydrogen-ion concentration, *Helminthosporium victoriae* toxin production, effect, 453; **illus. 457**
effect on *Phytophthora parasitica* var. *nicotianae* isolates, 346
poinsettia root rot, 552; **illus. 553**
soil, *Rhizoctonia solani*, effect, 466
8-Hydroxyquinoline benzoate, see under Chemotherapeutants
sulphate, see under Fungicides

Ichikawa, S. T., 576
Indole acetic acid, potato virus Y effect, 148
Inheritance of resistance, barley to net blotch, 174
cabbage, to blackrot, 35; **illus. 36**
cowpea to cucumber mosaic virus, 563
cucumber to mosaic, varieties, 451
to scab, varieties, 451
oats to crown rust, 219; **illus. 220**
Helminthosporium blight, 219; **illus. 220**
potato varieties to late blight in South America, 247
*Solanum* spp. to *Phytophthora infestans* races, breeding, 464
strawberry to Verticillium wilt, 387; **illus. 388**
tobacco to black shank, varieties, 463
to Fusarium wilt, varieties, 463
to mosaic virus, varieties, 463
to wildfire, varieties, 463
tomato to late blight, 103, 186
*Phytophthora infestans* races, breeding, 464
*Venturia inaequalis* to fungicides, tests, 467; **illus. 469**
Insecticides, general, rose blackspot, fungicide, miticide combinations, 465
Insecticides, aldrin, stem nematode, alfalfa, 348
malathion, rose blackspot, fungicide combination, 465
parathion, stem nematode, alfalfa, 348
Insects, general, larvae, sweet potato internal cork phellem diagnostic symptom, 290
virus assay techniques, review, 196, 208
Insects, *Agathis stigmaterus*, 37
aphid, *Amphorophora rubi*, strawberry vein banding virus, 307
*Aphis gossypii*, cucumber southern mosaic virus, 217
*rumicis*, cucumber southern mosaic virus, 217
bean, cucumber southern mosaic virus, 217
black peach, crown gall, 349
*Capitophorus fragaefolii*, strawberry vein banding virus, 307
cotton, cucumber southern mosaic virus, 217
green peach, cucumber southern mosaic virus, 217
*Macrosiphum granarium*, barley and oats yellow dwarf virus, 695
*pelargonii*, strawberry vein banding virus, 307
*pisi*, pea streak virus, 665, **illus.**
*Myzus ornatus*, strawberry vein banding virus, 307
*persicae*, carnation mosaic virus, 464
cucumber southern mosaic virus, 217
lettuce mosaic transmission, 357; **illus. 368**
tomato virus Y transmission, 148
yellow-net virus, 579, **illus.**
*Rhopalosiphum prunifoliae*, barley and oats yellow dwarf virus, 695
*Diatraea saccharalis*, yeast associated with, antibiosis, sugar cane rot, 37
flea beetle, tomato anthracnose, 340, **illus.**
leafhopper, *Collodonus geminatus*, cherry buckskin virus, 694
*Collodonus geminatus*, peach yellow leaf roll virus, celery, 694
*Macrosteles fascifrons*, celery-yellows virus transmission tests, 185
mites, *Aceria ficus*, fig mosaic transmission, 52, **illus. 54**
*Aceria tulipae*, wheat streak mosaic virus, spread, control, 116, **illuss. 117-120**
Iodoacetic acid, antifermentative for cultures of *Panagrellus redivivus*, 184
*Ipomoea tricolor* for sweet potato internal cork assay, 185

Jacobson, Louis, (43)
Jeffers, W. F., (466)
Jensen, D. D., 327, 694
Jimson weed, tobacco anthracnose in, 465
John, C. A., 636
Johnson, Folke, 110
Johnson, Gestur, (626)
Jones, Fred Reuel, 289
Jones, Jack E., (349)
Jones, Julian, (349)
Judy, T. M., (466)

Kaesberg, Paul, (145), (187)
Kahn, Robert P., 334, 500
Karathane, rose black spot, 465
Keitt, G. W., (467)
Keller, John R., 552
Kelman, Arthur, 348, (537)
Kenknight, Glenn, 348, 349
$\alpha$-Ketoglutaric acid effect on TMV multiplication, 291
Kingsland, G. C., 185
Kirkpatrick, Hugh C., 292, (574)
Klemmer, H. W., 618
Klotz, L. J., (517), (693), 694
Kofranek, A. M., (156)
Krupka, L. R., 465
Krusberg, Lorin R., 349

*Lactuca sativa*, see lettuce
spp., *Marssonina panattoniana* in, 375; **illuss. 377, 379**
Laird, Edward F., Jr., (595)
Lane, Susan A., 185
Latterell, Frances M., (500)
Leach, Charles M., 94, 405
Lebeau, J. B., 667
Leben, Curt, 467, (518)
Legume seed, pathogen isolation from, method, 94; **illuss. 95, 96**
Lemon inoculation method, 517, **illuss.**
tristeza, yellows and pitting complex, 222
*Leptosphaeria avenaria* pycnidial stage in oats, 278; **illuss. 279, 280**
*Lethum australiense* elimination in dahlias, 224; **illus. 225**
Lettuce anthracnose, etiology, host range, 375; **illus. 377, 379**
mosaic virus, aphid transmission, 357; **illus. 368**
seed transmission, 63; **illuss. 65, 67**
root rot, organisms and conditions associated with, 184
tobacco anthracnose in, 465
Light effect on aphid transmission of lettuce mosaic virus, 357
fungi, response to, 465
*Physalospora obtusa* effect on pycnidia production, 22; **illus. 23**
*Sclerotinia trifoliorum* apothecial production, 185
sugar beet yellows transmission, 233
wheat bunt resistance, 428

Lily, Croft, propagation, pathogen-free stocks, 693
leaf scorch and root rot, nutrition, control, 156; **illus. 159**
LINDBERG, GEORGE D., (145)
LINDNER, R. C., 574
LIVINGSTON, J. E., (503)
LORDELLO, LUIZ GONZAGA E., 465
LOWNSBERY, B. F., 163
LUCAS, G. B., (459), 537
LUKE, H. H., 453
LUND, STEVE, 181
LUNT, O. R., (156)
*Lycopersicon esculentum*, see Tomato
*Lycopersicum esculentum* var. *cerasifolium* resistance to tomato late blight, 186
*Lycopodium* sp., quantitative cyclone spore collector method, 240

*Macrosiphum* spp., see under Insects, aphid
*Macrosteles fascifrons*, celery yellows transmission tests, 185
Magnesium, see under Nutrition of microorganisms
MAGILL, MARY A., (295)
MAI, W. F., (184)
Maize, *Sclerospora sorghi* in, India, 128; **illus. 130**
Maleic hydrazide, see under Fungicides
Maneb, see under Fungicides
Manganous ethylenebis [dithiocarbamate], see Fungicides, maneb
Mangel Fusarium wilt, beet, relationships, 346
Manns, Thomas Franklin, biographical sketch, 241; **illus.**
Maple wilt, soil and sapwood water content, relation, 184
toxins produced by fungus, 183
MARAMOROSCH, KARL, 185
Marigold crown gall, antibiotics, inhibition, 618; **illuss. 621, 623, 624**
MARKLEY, BRIAN B., (693)
*Marmor tritici*, electron microscopy, 348
*tritici*, a winter rye for identifying, 637
*upsilon*, potato varieties reaction to, 148
*virgatum*, mite spread in wheat, 116; **illuss. 117-120**
MARSHALL, BARTON H., JR., 465, (465), 676
*Marssonina panattoniana*, lettuce, etiology, host range, 375; **illuss. 377, 379**
MARTIN, G. C., (26)
MARTIN, J. P., (694)
MARTIN, MARY, 407
MARTIN, W. J., 349
MARVEL, M. E., (103)
MCBETH, C. W., (576)
MCCALLAN, S. E. A., 295
MCCLEAN, A. P. D., 222
MCCLELLAN, W. D., 465
MCKEEN, W. E., 629
MEINERS, JACK P., 59
*Melampsora lini*, host-parasite interaction, 680
*Meloidogyne* spp., see under Nematodes
MENZIES, J. D., 527
Mercury chlorides, see under Fungicides
Methionine and tobacco wildfire toxin, 659; **illuss. 660-663**
Methyl bromide (bromomethane), see under Fungicides and under Soil fumigants
Methyl naphthoquinone, effect on spore germination, *Puccinia graminis*, 259; **illus. 265**
MIDDLETON, JOHN T., 694
MILBRATH, J. A., (696)
Miles, Gilbert Frazer, biographical sketch, 1, **illus.**
MILLER, LAWRENCE P., (295)
MILLER, PATRICK M., 461
MILLER, V. L., (110)
Millet, wheat streak mosaic virus, mite spread, 116
MILLIKAN, D. F., 565
MITCHELL, A. E., 185
Miticides, general, rose blackspot, fungicide, insecticide combinations, 465
Miticides, aramite, rose blackspot, fungicide combination, 465
ovotran, rose blackspot, fungicide combination, 465
*Monilinia fructicola*, light, response to, 465
MOORE, E. L., 349
MORGAN, O. D., 465
MORTON, D. J., 417
Moth borer, see Insects, *Diatraea saccharalis*
Muncie, Jesse Howard, biographical sketch, 523, **illus.**
MUNNECKE, DONALD E., (613), 694
Mushroom, *Ditylenchus* sp. in, 346
nematode injury, 346
Muskmelon Fusarium wilt, 435; **illus. 436**
*Myzus* spp., see under Insects, aphids

N-244, see under Nematocides
NATTI, JOHN J., 185
NELSON, R. R., 352, 639
Nemagon, see Nematocides, 1,2-dibromo-3-chloropropane, 576; **illus. 577**
Nematocides, Aabulba, 347
Aaventa, 347
benzene hexachloride, apple "pout", 477; **illuss. 477, 478**
*Xiphinema* sp. peach and apple, 464, 477; **illuss. 477, 478**
D-D (1,3-dichloropropene; 1,2-dichloropropane), tobacco, 349
tobacco root-knot, 349
1,2-dibromo-3-chloropropane (nemagon), soil diffusion, 576; **illus. 577**
Dowfume W-85 (1,2-dibromomethane), tobacco, 349
tobacco root-knot, 349
Heptachlor (1,4,5,6,7,8,8, heptachloro-3a, 4,7,7a-tetrahydro-4, 7-methanoindene) turf, nematode, 350
N-244 (3-*p*-chlorophenyl-5-methylrhodanine) turf, nematode, injury and control, 350
PN-20 (20% 1,2-dibromomethane in Phillips hydrocarbon mixture), tobacco, 349
R-I-26 (an unsaturated aliphatic compound), turf, nematode, 350
V-C 1-13 (a neutral ester of phosphorothioic acid), turf, nematode, 350
Nematodes, general, citrus, control, 693
control, organic mercury dips, 347
cotton Fusarium wilt studies, 349
estimating population method, 238; **illus. 238**
injury to mushroom in absence of microorganisms, 346
nemagon soil diffusion, 576; **illus. 577**
turf, control, 350
Nematodes, *Criconemoides* sp. pathogenicity in tobacco and Spanish peanuts, 347
*Ditylenchus dipsaci* in alfalfa, control, 348
sp. in mushroom, 346
ectoparasitic, strawberry root rot and decline, control, 183
*Helicotylenchus*, boxwood, 464
sp. in cotton, 349
spp., tobacco, control, 349
*Heterodera rostochiensis* control, 347
*rostochiensis*, host and non-host stimulation of larval emigration, 184
*tabacum* effect on growth of tobacco, control, 163
*Hoplolaimus coronatus* in cotton, 349
*Meloidogyne arenaria* perineal patterns, **illus. 29**; 34
*arenaria*, tobacco rotation, 540; **illuss. 543, 544**
*thamesi* perineal patterns, **illus. 30**; 34
*brevicauda* perineal patterns, **illus. 33**; 34
*exigua* perineal patterns, **illus. 33**; 34
*hapla* perineal patterns, **illus. 28**; 34
soybean resistance, 347
*incognita* perineal patterns, **illus. 31**; 34
*acrita*, alfalfa, 70; **illus. 71**
cotton, 540; **illuss. 543, 544**
cotton Fusarium wilt, 349
perineal patterns, **illus. 32**; 34
soybean resistance, 347

Nematodes, *Meloidogyne incognita acrita—continued*
tobacco Granville wilt, 537; **illuss. 538, 539**
rotation, 540; **illuss. 543, 544**
*javanica*, alfalfa, 70; **illus. 71**
perineal patterns, 26; **illuss. 26, 27**
sp. in cotton, 349
tobacco, control, 349
resistance, 349
spp. control, 347
nemagon, soil diffusion, 576; **illus. 577**
perineal patterns, 26, **illuss. 27-33**
soybean, Brazil, 465
tobacco black shank relationship, 459; **illus. 460**
*Panagrellus redivivus*, antifermentative chemicals for culture of, 184
*Paratylenchus minutus*, estimating populations, 238; **illus. 238**
*Pratylenchus* sp. in cotton, 349
sp., tobacco resistance, 349
spp. control, 347
tobacco, control, 349
root-knot, see also Nematodes, *Meloidogyne*
alfalfa, 70; **illus. 71**
seasonal fluctuations, tobacco, **illus. 543**
soybean resistance, 347
structure, function and host, 466
sweet potato internal cork, phellem, diagnostic symptom, 290
*Rotylenchus brachyurus* pathogenicity in tobacco, 347
*Trichodorus* sp. in cotton, 349
*Tylenchorhynchus claytoni*, tobacco, control, 349
*claytoni*, tobacco resistance, 349
sp. in cotton, 349
*Xiphinema americanum*, tobacco, control, 349
sp., apple and peach, control, 464, 477; **illuss. 477, 478**
Neomycin, soil production and role, 407
NEWSOM, L. D., (349)
*Nicotiana glutinosa*, mosaic virus, chemicals in leaves, 43; **illus. 45**
*glutinosa*, potato virus X, nutrition, 567; **illus. 570**
potato virus X in, temperature effect, 493; **illuss. 494-496**
TMV, calcium deficiency effect, 288
on lesions, 695
temperature of infection effect, 595
time course studies, 695
*tabacum*, see Tobacco
NIELSEN, L. W., (290)
Nitrogen, see under Nutrition and Nutrition of microorganisms
*Nocardia formica*, antibiotic from, antiviral effect, 281
Noformicin, see also under Antibiotics
bean southern mosaic virus, 281
tobacco mosaic virus, 281
Nomenclature, fungicides, 295
NOUR-ELDIN, FARID, 291
Nub-root, cotton, 413; **illus. 415**
flax, 413; **illus. 415**
NUSBAUM, C. J., 349, (540)
Nutrient sprays, tomato Fusarium wilt, 443
Nutrition, calcium, TMV, deficiency effect on *Nicotiana glutinosa*, 288
calcium chloride, tomato Fusarium wilt, spray, 443
lilies Rhizoctonia rot and injury, 156; **illus. 159**
nitrogen, lily Rhizoctonia rot and injury, 156; **illus. 159**
potato X, *Nicotiana* spp., 567; **illus. 570**
tobacco ring spot, cucumber, 567; **illus. 570**
tomato Fusarium wilt, sprays, 443
phosphorus, potato X, *Nicotiana* spp., 567
tobacco ring spot virus, cucumber, 567
potassium chloride, tomato Fusarium wilt, spray, 443
potato X, *Nicotiana* spp., 567; **illus. 570**
sodium chloride, tomato Fusarium wilt, spray, 443

Nutrition—*continued*
tobacco ring spot virus, cucumber, 567; **illus. 570**
tomato Fusarium wilt, sprays effect, 443
Nutrition of microorganisms, biotin, *Sclerotinia trifoliorum*, 39
boron, *Stromatinia gladioli*, 465
*Botrytis gladiolorum*, 676; **illus. 677**
carbon sources, basidiomycete, low temperature, **illus. 668**
*Fusarium oxysporum* var. *nicotianae*, 350
*Curvularia lunata*, 676; **illus. 677**
*Fusarium oxysporum* f. *gladioli*, 676; **illus. 677**
magnesium, *Stromatinia gladioli*, 465
nitrogen sources, basidiomycete, low temperature, **illus. 668**
*Fusarium oxysporum* var. *nicotianae*, 350
*Sclerotinia trifoliorum*, 39
*Stromatinia gladioli*, 465
phosphorus, *Stomatinia gladioli*, 465
potassium, *Stromatinia gladioli*, 465
*Sclerotinia trifoliorum*, 39
*Stemphylium* sp., 676; **illus. 677**
*Stromatinia gladioli*, 676; **illus. 677**
sclerotia production, 465
vitamin B8, *Sclerotinia trifoliorum*, 39
NYLAND, GEORGE, 694

Oak stump solution intake, 466
trunk canker, *Polyporus hispidus*, 177; **illuss. 179, 180**
wilt fungus longevity under mineral oil, 405
mat formation studies, 485; **illuss. 487, 488**
transmission by squirrels, 581; **illus. 582**
Oats, black stem phase of Septoria disease, 278
calcium sulfamate effect, 503
crown rust control, 410
reaction, 462; **illus.**
resistance to, 275
germ plasm, 219; **illus. 220**
sources of, method, 410
Helminthosporium blight, resistance, germ plasm, 219; **illus. 220**
*victoriae* toxin production, 453; **illuss. 456, 457**
root-knot nematode, **illus. 543**
*Septoria avenae* in, moisture and temperature effect, control, 181; **illus. 182**
disease, 278; **illuss. 279, 280**
stem rust control, 503
resistance, X-ray, mutation, 490
yellow dwarf virus, Holland, 695
OCHOA, C., 247
OGAWA, JOSEPH M., 239, 695
OHMS, R. E., 513
Onion pink root hosts, 350
Orange inoculation method, 517, **illuss.**
tristeza, yellows and pitting complex, 222
Orchid Fusarium wilt, 599; **illuss. 600, 601**
*Oryzopsis hymenoides*, wheat streak mosaic mite spread, 116
OSWALD, J. W., (428), (693), 695
OWEN, JOHN H., 435
Oxyquinoline benzoate, antifermentative for cultures of *Panagrellus redivivus*, 184

PALMITER, D. H., 186
*Panagrellus redivivus*, antifermentative chemicals for culture of, 184
Panogen, barley scald, 350
PANTIDOU, MARIA E., 338
*Paratylenchus minutus*, estimating populations, 238; **illuss. 238**
Parathion, see under Insecticides
PARKER, K. G., (186)
PARRACK, A. L., (49)
Patulin, see under Antibiotics
PAULUS, ALBERT OTTO, 168

Pea, *Macrosiphum pisi*, streak virus, 665, **illus.**
root rot, *Fusarium solani*, control, 185
streak virus, electron microscopy, **illus. 189**
tobacco anthracnose in, 465
Peach, apple mosaic virus, 292; **illus. 293**
blackberry mosaic virus, transmission to, 272
brown rot control, 347, 695
crown gall, black peach aphid, 349
"mule ear" virus disorder, 323; **illuss. 325, 326**
Rhizopus control, 347
rosette virus, antibiotics, chemotherapy, 348
*Prunus tomentosa* index plant, 320; **illuss. 321-323**
scab control, 347
spring canker, time of infection related to, 285
storage rot control, 347
western X disease, yellow leaf roll, interference, 694
*Xiphinema* sp. in, control, 464, 477; **illuss. 477, 478**
yellow bud mosaic virus, soil, retention by, 696
yellow leaf roll virus, celery host, 694
leafhopper transmission, celery, 694
western X, interference, 694
Peanut, *Pseudomonas solanacearum*, pathogenicity of isolates, 348
root-knot nematode, **illuss. 543, 544**
Spanish, *Criconemoides* sp. pathogenicity on, 347
Pear blast, *Pseudomonas syringae*, control, 629; **illus. 630**
blossom blast, boron deficiency, control, 110; **illuss. 111, 113**
twig dieback, boron deficiency, control, 110; **illuss. 111, 113**
Pectic enzyme, see under Enzymes
Pectin methylesterase, see under Enzymes
*Pelargonium* spp. bacterial stem and leaf disease, 613; **illus. 616**
Penicillin, *Agrobacterium tumefaciens* and gall tissue, 618; **illus. 623**
*Penicillium* in cotton seedlings, control, 350
fungus culture, 466
*martensii* in asparagus, symptoms and control, 527; **illus. 528**
*oxalicum*, filipin, assay, 559
spp., citrus inoculation method, 517, **illuss.**
*Peperomia*, nematode control, 347
Pepper bacterial leaf spot, control, 183, 465
*Cercospora sapsici*, resistance, 347
cucumber mosaic virus, aphid-vector studies, 217
*Stemphylium* **floridanum** in, 11
tobacco anthracnose in, 465
*Peronospora effusa*, spinach resistance, screening plants for, 635
Person, L. H., (348)
Peru, potato wild species and breeding, 247
*Pestalotia* sp., light, response to, 465
Peters, B. G., (163)
Peterson, C. E., (313), (445)
*Petunia hybrida*, see Petunia
*Stemphylium* **floridanum** in, 11
*Phaseolus vulgaris*, see Bean
Phellem, sweet potato internal cork, diagnostic symptom, 290
Phenolic compounds, *Streptomyces scabies*, 626; **illus. 627**
Phenyl mercury acetate, see Fungicides, PMAS
*Philodendron hastatum*, physiologic leaf spot, control, 694
nematode control, 347
*Phleum pratense*, see Timothy
Phosphorus, see under Nutrition of Microorganisms
Photoperiod, barley Pythium root rot, 97; **illus. 100**
*Physalis* spp. yellow-net virus, 579, **illus.**
*Physalospora obtusa* in apple and control, 392; **illus. 393, 395**
*obtusa*, light effect on pycnidia production, 22; **illus. 23**
medium for, 461
*tucumanensis*, *Candida intermedia*, antibiosis, 37
*Phytophthora aphanidermatum*, swarming, 694
*capsici* mating with tobacco isolates, *P. parasitica*, 346
Phytophthora—*continued*
*cinnamomi*, fungicides, tests, 398; **illus. 401**
*citrophthora*, swarming, 694
*infestans*, medium for, 461
physiologic races, use in breeding, 464
potato breeding for resistance, review, 250
races, potato to tomato, 473
*Solanum demissum*, histology, 546; **illuss. 549, 551**
spp., breeding for resistance, South America, 247
temperature effect on germination, 673; **illuss. 674, 675**
tomato inheritance of resistance, 103, 186
*parasitica*, swarming, 694
var. *nicotianae* isolate comparisons, 346
nematode relationship to tobacco resistance, 459; **illus. 460**
pathogenicity, 346
resistant varieties, 463
tobacco resistance, 348
spp. on wild plants, 694
Pierson, C. F., (451), 524
Pine, T. S., 267
Pineapple, chloride injury and control, 590; **illuss. 591, 592**
sea water injury and control, 590; **illus. 591, 592**
Pleocidin, see under Antibiotics
*Pleospora herbarum*, medium for, 461
*lycopersici* in tomato fruit tumor, 136
*phaeocomes* in orchard grass, 633, **illus.**
PN-20, see under Nematocides
*Poa compressa*, wheat streak mosaic, mite spread, 116
Poinsettia root rot, 552; **illuss. 553, 555, 557**
Polley, Dorothy, (110)
Polygalacturonase, see under Enzymes
Polymyxin, see under Antibiotics
*Polyporus hispidus*, oak trunk canker, 177; **illuss. 179, 180**
Popp, W., 585
Porter, R. H., 637
Potassium, see under Nutrition and Nutrition of Microorganisms
Potato, breeding for yield, South America, 248
charcoal rot, India, 91
frost injury, breeding for resistance, South America, 247
golden nematode, stimulation of larval emigration, 184
internal brown spot associated with hot dry weather, 184
late blight, breeding for resistance, review, 250
breeding for resistance, South America, 247
leafroll virus, reaction of starch from cells, 185
*Phytophthora infestans*, histology, 546; **illuss. 549, 551**
races, tomato, relationship, 473
*Pseudomonas solanacearum*, pathogenicity of isolates, 348
scab, phenolic compounds, inhibition, 626; **illus. 627**
soft rot, suberin and periderm formation, 649; **illuss. 651-653**
*Stemphylium* **floridanum** in, 11
virus S, latent virus related to, 693
virus X immunity, 445; **illuss. 447-449**
immunity, clonal lines, 313; **illuss. 316, 318**
latent virus related to S, 693
nutrition, *Nicotiana* spp., 567; **illus. 570**
temperature effect in *Nicotiana* spp., 493; **illuss. 494-496**
virus Y, varieties reaction to, 148
yellow-dwarf virus, techniques, review, 208; **illus. 214**
*Pothos*, nematode control, 347
Pound, Glenn S., (168), 493, (567)
Powdery mildew, beans, chemicals in leaves, 43, **illus. 45**
Powell, Dwight, (285)
Powers, H. R., Jr., (459)
*Pratylenchus* spp., see under Nematodes
Protopectinase, see under Enzymes
Prune dwarf virus, *Prunus tomentosa* index plant, 320; **illuss. 321-323**

*Prunus americana*, X-disease, anthocyanosis and carbohydrate content of leaves, 184
*domestica*, X-disease, anthocyanosis and carbohydrate content of leaves, 184
*mahaleb*, *P. tomentosa*, virus index plant, 320; **illus. 323**
ring spot virus, 565
*sieboldi*, X-disease, anthocyanosis and carbohydrate content of leaves, 184
spp. X-disease, anthocyanosis and carbohydrate content of leaves, 184
*tomentosa*, cherry viruses index plant, 320; **illuss. 321-323**
*virginiana*, see Chokecherry
*Pseudomonas phaseolicola*, resistance to, testing method, 519; **illus. 520**
*solanacearum*, pathogenicity of isolates, different hosts, 348
root-knot nematode in tobacco, 537; **illuss. 538, 539**
tobacco, composition of leaves, 506
spp., *Bacterium herbicola*, antagonsims, 466
*Sclerotium rolfsii* effect, **illus. 418**
*syringae*, pear blast, 629; **illus. 630**
*tabaci*, resistant varieties, 463
toxin, mode of action, 659; **illuss. 660-663**
*Pseudoplea briosiana*, *Stemphylium botryosum*, relationship, 352
*chrysanthemi* distribution, 644
temperature, 644; **illuss. 645-647**
*Puccinia coronata* control, 410
*coronata*, oats, resistance, sources of, method, 410
var. *avena*, oats reaction, 462, **illus.**
oats resistance, 275
*glumarum*, races in South America, 245
*graminis avenae*, oats resistance, X-ray mutation, 490
control in bluegrass, 186
f. *tritici* control, 503
heterocaryosis basis for variation, 639; **illus. 641, 643**
races in South America, 242; **illus. 243**
self-inhibition, role in germination, 259; **illuss. 260, 261, 263, 265**
*polygoni-amphibii*, chlorophyll relationship in parasitism, 186
*sorghi*, chlorophyll relationship in parasitism, 186
spp. races in South America, 242
*triticina*, races in South America, 242; **illus. 244**
Puratized Agricultural spray, see under Fungicides
PURDY, LAURENCE H., 421
Pycnidial production, light effect, *Physalospora obtusa*, 22; **illus. 23**
*Pyracantha*, streptomycin translocation, 692
tetracycline translocation, 692
*Pyrenochaeta terrestris*, actinomycetes antagonism, 440; **illuss. 441**
*Pythium arrhenomanes*, barley adaptation, 97; **illuss. 98, 100**
cotton seedlings, control, 350
sp., tomato damping-off control, 347
spp., lettuce root rot, associated with, 184
nub-root, cotton and flax, 413
poinsettia root rot, 552; **illus. 555**
wild plants, 694

*Quercus falcata* var. *pagodaefolia*, *Polyporus hispidus*, trunk canker, 177
*nigra*, *Polyporus hispidus*, trunk canker, 177
*nuttalli*, *Polyporus hispidus*, trunk canker, 177; **illuss. 179, 180**
*phellos*, *Polyporus hispidus*, trunk canker, 177; **illuss. 179, 180**
spp., see also Oak

RAABE, ROBERT D., 55, 695
Radioisotopes, $C^{14}$ accumulation in diseased leaves, 43; **illus. 46**
Radioisotopes—*continued*
$P^{35}$ accumulation in diseased leaves, 43; **illus. 46**
$S^{35}$ accumulation in diseased leaves, 43; **illus. 45**
RAPPAPORT, I., 695
Raspberry, blackberry mosaic virus, transmission to ,272
REED, H. E., 350
Resistance, alfalfa to root-knot, 70; **illus. 71**
barley to net blotch, inheritance, 174
bean to bacterial blights, testing method, 519; **illus. 520**
cabbage to blackrot, 55
blackrot, inheritance of, 35; **illus. 36**
cowpea, Brown Sugar Crowder, to Fusarium wilt, 347
to cucumber mosaic virus, 563
cucumber to mosaic, varieties, 451
to scab, varieties, 451
oats to crown rust, 219; **illuss. 220, 275**
to crown rust, sources of, method, 410
to Helminthosporium blight, 219; **illus. 220**
to stem rust, mutation, X-ray, 490
pepper to Cercospora, 347
potato to frost injury, South America, 247
to late blight, breeding South America, 247
to *Phytophthora infestans*, histology, 546; **illuss. 549, 551**
*Solanum* spp. to *Phytophthora infestans* races, breeding, 464
soybean to root knot nematode, 347
spinach blight, screening for, 635
blue mold, screening plants for, 635
strawberry to Verticillium wilt, 387; **illus. 388**
tobacco to black shank, 348, 349
to black shank, nematode relationship, 459; **illus. 460**
varieties, 463
to Fusarium wilt, 348
varieties, 463
to mosaic virus, 463
to nematodes, 349
to wildfire, varieties, 463
tomato to Fusarium, 303
late blight, inheritance, 103, 186
to *Phytophthora infestans* races, breeding, 464
*Venturia inaequalis* to fungicides, tests, 467; **illus. 469**
wheat to bunt, environmental factors, 428; **illus. 430, 432, 433**
to loose smut, 8
reaction, 513; **illus. 514**
REYNOLDS, HAROLD W., 70
*Rhaphiolepis* spp., see Hawthorn
*Rhizoctonia* cotton seedling, control, 350
*solani* control in gladiolus, 693
control, soil factors and fungicides, 466
Croft lilies, nutrition, control, 156; **illus. 159**
effect of $CO_2$ on distribution in host, 693
nub-root, cotton and flax, 413
tomato damping-off control, 347
turf, control, 186
sp., *Bacillus subtilis*, antagonistic, 252; **illuss. 255, 257**
spp. poinsettia root rot, 552
tomato fruit tumor, 136
Rhizopus in cotton seedlings, control, 350
*Rhopalosiphum prunifoliae*, barley and oats yellow dwarf virus, 695
*Rhus typhina*, see Sumac
*Rhynchosporium secalis* in barley, control, 350
R-I-26, see under Nematocides
RICE, ROBERT V., 145
RICH, A. E., (185), 186
RICHARDS, M. C., (186)
RIKER, A. J., (618)
ROHDE, R. A., (673)
ROISTACHER, C. N., 517
ROMANKO, R. R., 466
Root-knot, see under Nematodes
ROSBERG, DAVID W., 49

Rose blackspot control, 465
Verticillium wilt, 695
ROSEN, H. R., 219
ROSS, ELDON, (466)
*Rotylenchus brachyurus* pathogenicity in tobacco, 347
ROWELL, J. B., 370
Rubrofusarin, *Fusarium oxysporum* var. *nicotianae*, production by, 350
RUSHDI, M. H. K., 466
Rust, bean, chemicals in leaves, 43; **illuss. 45, 46**
crown, oats, reaction, 462, **illus.**
resistance, 219; **illus. 220, 275**
stem, bluegrass, control, 186
wheat, races in South America, 242; **illuss. 243, 244**
Rye, wheat streak mosaic virus, mite spread, 116
a winter, wheat mosaic virus, identifying, 637

*Saccharomyces*, fungus culture, 466
*pastorianus*, filipin, 559
SAFEEULLA, K. M., 128
*Samolus parviflorus* curly top virus, reaction and recovery, 531; **illuss. 532, 535**
*Sansevieria*, nematode control, 347
SASSER, J. N., (349), 459, (537), 540
SAYED, G. S., 186
SCHAAL, LAWRENCE A., 626
SCHALLER, C. W., 174
SCHEFFER, R. P., (303), (381)
SCHROEDER, HARRY W., 288
SCHROEDER, W. T., (338)
SCHUSTER, M. L., 519
*Sclerophthora macrospora* in wheat, corn downy mildew compared, 350
*Sclerospora sorghi*, maize and sorghum, India, 128; **illus. 130**
*Sclerotinia fructicola* control, 695
*fructicola*, quantitative cyclone spore collector method, 239; **illus. 239**
*intermedia*, species concept, variability, 421; **illus. 425**
*minor*, species concept, variability, 421; **illus. 425**
*sativa*, sclerotium size, **illus. 425**
*sclerotiorum*, species concept, variability, 421; **illus. 425**
spp., sclerotium size, **illus. 425**
species concept, variability, 421; **illus. 425**
*trifoliorum* apothecial production, 185
physiological differences between strains, 39
species concept, variability, 421; **illus. 425**
*Sclerotium rolfsii* in gladiolus, methyl bromide, control and injury, 520
*rolfsii*, light, response to, 465
soil microorganisms, antagonistic, 417; **illus. 418**
thiamin, 417
SCOTT, H. A., (288)
*Secale cereale*, wheat mosaic virus, for identifying, 637
Seed counters, vacuum, **illus. 96**
disinfecting apparatus, **illus. 95**
drier, aseptic, **illus. 96**
maturation effect on bean southern mosaic virus, 17
pathogen isolation from, method, 94; **illuss. 95, 96**
transmission, bean southern mosaic virus, 18
treatment, barley loose smut, water-soak, 518
barley, water-soak, germination, effect, 518
Semesan, *Rhizoctonia solani*, 466
SENSENEY, C. A., (334)
*Septoria avenae* in oats, 279; **illuss. 279, 280**
*avenae*, oats, moisture and temperature effect, control, 181; **illus. 182**
*Puccinia coronata* var. *avena*, 462
*lycopersici*, medium for, 461
*Serratia marcescens*, *Sclerotium rolfsii* effect, **illus. 418**
Shallot pink root, actinomycetes antagonism, 440; **illuss. 441**
*Pyrenochaeta terrestris* in, 350
SHANDS, H. L., (181)
SHANKS, JAMES B., (552)
SHURTLEFF, M. C., 186
SIDERIS, C. P., 590
SIEGEL, A., 695
SILL, W. H., JR., 637
SIMONS, JOHN N., 217
SIMONS, M. D., 275, 410, 462
SINCLAIR, J. B., 563
SKOTLAND, C. B., 603, 665
SLYKHUIS, JOHN T., 116
SMART, HELEN F., (649)
SMITH, FLOYD F., (464), (465)
SMITH, WILSON L., JR., 88, 649
SMOOT, JOHN J., (673)
Smut, barley loose, varietal resistance, 73, **illus. 77**
wheat loose, inheritance of resistance, 8
SNOW, GORDON F., 696
Sodium, see also under Nutrition
benzoate, antifermentative for culture of *Panagrellus redivivus*, 184
diethyl dithiocarbamate, tomato resistance, effect, 303
fluoride, tomato Fusarium resistance, effect, 303
methyldithiocarbamate, see Fungicides, Stauffer N-869
sulfanilate, see under Fungicides
Soil Bacteria, antibiotics production and role, 407
conditions, lettuce root rot, associated with, 184
fumigation, gladiolus, 693
fumigants, DD, nitrogen in soil, tobacco, nematodes, 163
DD (dichloropropene-dichloropropane) tobacco growth, nematodes, 163
Dowfume W-85 (1,2-dibromoethane), strawberry root rot and decline, ectoparasitic nematodes, 183
methyl bromide, gladiolus, 693
moisture effect on *Septoria avenae* in oats, 181
*Rhizoctonia solani*, fungicides, factors effect, 466
temperature effect on potato virus X, *Nicotiana* spp., 493
*Verticillium albo-atrum* longevity in, 180
-water, maple *Verticillium* wilt, relation, 184
*Solanum aculeatissimum*, *Stemphylium* **floridanum** in, 11
*citrullifolium*, golden nematode, stimulation of larval emigration, 184
*demissum*, late blight, breeding for resistance, review, 250
*Phytophthora infestans*, histology, 546; **illuss. 549, 551**
*dulcamara*, golden nematode, stimulation of larval emigration, 184
spp. breeding South America, 247
golden nematode, stimulation of larval emigration, 184
late blight, breeding for resistance, review, 250
*Phytophthora infestans*, resistance to races, breeding, 464
*Stemphylium* **floridanum** in, 15
*tuberosum*, see Potato
*xantii*, golden nematode, stimulation of larval emigration, 184
SOMMER, NOEL F., 607
Sorghum, *Sclerospora sorghi* in, India, 128; **illus. 130**
South America, potato wild species and breeding, 247
wheat rust races, 242
SOVA, CARL, (636)
Sowbane, sugar beet yellows, mechanical transmission, 233; **illus. 234**
Soybean bud-blight, tobacco—and tomato ringspot virus, incitant, 500; **illus. 501**
*Meloidogyne* spp., in, Brazil, 465
*Pyrenochaeta terrestris* in, 350
root-knot nematode, resistance to, 347
tobacco anthracnose in, 465
Spergon, see Fungicides, chloranil
Spinach bacterial soft rot, control in packaged, 88
blight resistance, screening plants for, 635
blue mold, screening plants for, 635
Fusarium wilt, beet, relationships, 346
Spore germination, inhibition, *Puccinia graminis*, 259; **illuss. 260, 261, 263, 265**
isolation method, 405; **illus. 406**
SPRAGUE, RODERICK, 696

SPROSTON, THOMAS, (185)
Spruce canker control, 686
canker, *Valsa kunzei* relation to, 686; **illus. 688**
Squash mosaic virus, 3 components in, 145
mosaic virus, electron microscopy, **illus. 192**
sedimentation patterns, **illus. 146**
STAHMANN, MARK A., (145), 187, (303), (381), (524), (603)
Starch from cells of leafroll virus infected potatoes, 185
STARR, MORTIMER P., 613
Stauffer —, see under Fungicides
STERRE, RUSSEL L., 196
STEINER, G., 466
*Stemphylium botryosum*, alfalfa, 352; **illus. 353**
*botryosum*, *Pseudoplea briosiana*, relationship, 352
*S. callistephi*, *S.* **floridanum** conidia, **illus. 13**
*S. botryosum*, *S.* **floridanum** conidia, **illus. 13**
**floridanum** sp. nov., tomato leaf spot, 11; **illuss. 12, 13**
*solani*, histology in tomato, 654; **illuss. 655-657**
*S.* **floridanum** sp. nov. compared, 11; **illuss. 12, 13**
sporulation in culture, irradiation, 141; **illuss. 142, 143**
tomato, temperature effect, 168; **illuss. 169, 171**
sp., light, response to, 465
nutrition tests, 676; **illus. 677**
spp., grape decay, 229
medium for, 461
STEWART, ROBERT B., 413
Stone fruit ring spot virus, cherry, 565
virus, PLMV, purification, 574; **illus. 575**
Strawberry root rot and decline, ectoparasitic nematodes, control, 183
variegation, 464
vein banding virus, 307; **illus. 309**
Verticillium wilt resistance, 387; **illus. 388**
virus-free and non-virus-free study, 183
*Streptomyces* **filipinensis**, nov. sp., filipin from, 559; **illus. 560**
fungus culture, 466
*scabies*, phenolic compounds, inhibition, 626; **illus. 627**
Streptomycin, see under Antibiotics
Streptothricin, see under Antibiotics
*Stromatinia gladioli* control, 693
*gladioli*, nutrients effect on sclerotia production, 465
nutrition tests, 676; **illus. 677**
STROUBE, W. H., (417)
*Stysanus* sp., *Verticillium albo-atrum*, antagonistic, 696
Subtilin, soil, production and role, 407
Succinic acid effect on TMV multiplication, 291
Sugar beet damping-off, *Bacillus subtilis* antagonistic to pathogen, 252; **illuss. 255, 257**
Sugar cane mosaic virus, electron microscopy, 694
red rot, yeast antibiosis, 37; **illus. 38**
Sulfur, see under Fungicides
Sumac Fusarium wilt, aster relationships, 346
Sunflower, tobacco anthracnose in, 465
Sweet potato, internal cork virus, elimination, 185
internal cork, phellem, diagnostic symptom, 290
SYLVESTER, EDWARD S., 357

TAKAHASKI, WILLIAM N., 696
TAPKE, V. F., 73
TARJAN, A. C., (184), 350
Taxonomy, biological, coding, 58
TAYLOR, A. L., 26
TAYLOR, CARLTON F., 673
TAYLOR, EDGAR A., (465)
TAYLOR, JACK, 392
Techniques, autoradiograph, tobacco mosaic virus exposed to $Na_2S^{35}$, 44
bacteria and fungi, medium for, 461
bacteriophage of *Xanthomonas pruni*, production, 580
bean resistance to bacterial blights, testing, 519; **illus. 520**
Techniques—*continued*
breeding, *Phytophthora infestans* physiologic races, use in, 464
cherry viruses index plant, *Prunus tomentosa*, 320; **illuss. 321-323**
colorimetry, viruses, 196
dilution end-point, stream double refraction, 196
dry-weight determination, viruses, 196
electron microscopy, 187; **illuss. 189, 190, 192, 194; 196, 208**
apparatus for frozen-dried specimens, **illus. 190**
electrophoresis, zone, virus assay, 211
fungi and bacteria, medium for, 461
fungicides testing, lab., *Phytophthora cinnamomi*, 398; **illus. 401**
fungus culture conservation, method comparison, 466
gladiolus hot-water treatment, 693
grape in storage, forecasting decay, 229; **illuss. 230, 231**
inoculation of citrus, 517, **illuss.**
and incubation, *Phytophthora infestans* in *Solanum demissum*, 546
insect vector viruses, 196
wheat bunt, 480
techniques, insect vectors, virus assay, 208
longevity of *Endoconidiophora fagacearum* under mineral oil, 405
mechanical inoculation, viruses, 196
nematode culture, antifermentative chemicals for, 184
estimating population, 238; **illus. 238**
oats resistance to crown rust, sources of, 410
pathogen isolation from seed, 94; **illuss. 95, 96**
purification, viruses, 196, 208
seed counters, vacuum, **illus. 96**
disinfecting apparatus, **illus. 95**
drier, asceptic, **illus. 96**
serology, viruses, 196
sexual compatibility, *Ustilago zeae*, 370; **illus. 372**
spectrophotometry, viruses, 196
spinach blight and blue mold resistance, screening for, 635
spore collector, quantitative cyclone, 239; **illus. 239**
isolation device, 405; **illus. 406**
stone fruit virus, PLMV, purification, 574; **illus. 575**
stream double refraction, dilution end-point, 196
susceptible strawberry plant elimination, Verticillium wilt, 387
toxin production, *Helminthosporium victoriae* bioassay, 456
*Ustilago nuda* physiologic races study, 73
viruses, assay, review, 196
insect-transmitted, review, 208
sources, 209
wheat mosaic virus, a winter rye for identifying, 637
Tehon, Leo Roy, biographical sketch, 115, **illus.**
Temperature effect on *Alternaria tomato*, 168; **illuss. 169, 171**
aphid transmission of lettuce mosaic, 357
oak wilt mat formation, 485; **illus. 487**
oats crown rust resistance, 219; **illus. 221**
Helminthosporium blight resistance, 219; **illus. 221**
light, pycnidia production, *Physalospora obtusa*, effect, 24
*Phytophthora infestans* germination, 673; **illuss. 674, 675**
poinsettia root rot, 552; **illuss. 553, 557**
potato internal brown spot, associated with, 184
soft rot, suberin and periderm formation, 649; **illuss. 651-653**
virus X, 313; **illus. 316**
*Nicotiana* spp., 493; **illuss. 494-496**
symptoms; 445; **illuss. 447-449**
*Puccinia chrysanthemi*, 644; **illuss. 645-647**
*Septoria avenae* in oats, 181
*Stemphylium solani* in tomato, 168; **illuss. 169, 171**
tobacco mosaic virus, 595

Temperature—*continued*
tomato fruit tumor, **illus. 135**
wheat bunt inoculation, 480
resistance, 428; **illus. 432**
walnut branch-wilt, 607
Terramycin, see under Antibiotics
Tersan, see Fungicides, thiram
Tetrachloro-*p*-benzoquinone, see Fungicides, chloranil
Tetrachloronitrobenzene, grape injury in storage, 137
Tetracycline, *Pyracantha*, translocation, 692
Tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione, see Fungicides, Stauffer N-521
Tetramethylthiuram disulfide, see Fungicides, thiram
Thiamin, *Sclerotium rolfsii*, 417
Thiamine effect on wheat bunt inoculation, 480
*Thielaviopsis basicola*, poinsettia root rot, 552; **illuss. 553, 555, 557**
*basicola*, resistant tobacco varieties, 463
Thiolutin, see under Antibiotics
Thiouracil, bean southern mosaic virus, effect, 281
tobacco mosaic virus, effect, 281
Thiourea, tomato Fusarium resistance, effect, 303
Thiram, see under Fungicides
THIRUMALACHAR, M. J., 91, (128)
THOMAS, H. EARL, (272), (694)
THUNG, T. H., (695)
*Tilletia caries* in wheat, chlamydospore formation, **illus. 432**
*caries*, wheat inoculation methods, 480
wheat resistance, environmental factors, 428; **illuss. 430, 432, 433**
spp., *Gibberella zeae* antagonism, 288
*Helminthosporium sativum* antagonism, 288
TIMIAN, ROLAND G., 313, 445
Timothy Helminthosporium leaf streak, 227; **illus. 227**
TIMS, E. C., 350, (440)
TISDALE, W. H., 1
Tobacco anthracnose host range, 465
bacterial wilt, composition of leaves, 506
black shank fungus pathogenicity, 346
isolate comparisons, 346
nematode relationship, 459; **illus. 460**
resistance, 348, 349
varieties, 463
carnation ringspot virus, 464
*Criconemoides* sp. pathogenicity on, 347
cyst nematode, effect on growth, control, 163
Fusarium wilt, composition of leaves, 506
nutrition and metabolism, 350
resistance, 348
varieties, 463
Granville wilt, root-knot nematode, 537; **illuss. 538, 539**
mosaic virus, anomalous proteins associated with, 696
assay techniques, 196
calcium deficiency effect on, 288
chemical accumulation in leaves, 43; **illus. 45**
Cymbidium mosaic, compared in *Datura stramonium*, **illus. 331**
early time course of, 695
electron microscopy, **illuss. 189, 192, 194**; 603; **illuss. 604-606**
*Nicotiana glutinosa*, 695
noformicin effect, 281
organic acids effect on, 291
resistant varieties, 463
strains, 696
temperature of infection, effect, 595
thiouracil effect, 281
tomato seed, 636
nematodes, control, 349
resistance, 349
potato virus X, nutrition, 567; **illus. 570**
temperature effect, 493; **illuss. 494-496**
*Pseudomonas solanacearum*, pathogenicity of isolates, 348
Tobacco—*continued*
ringspot virus assay techniques, 196
composition of leaves, 506
nutrition, cucumber, 567; **illus. 570**
soybean bud-blight incitant, 500; **illus. 501**
root-knot nematode-black shank relationship, 459; **illus. 460**
control, 349
rotation, season, effects, 540; **illuss. 543, 544**
root-rot, black, resistant varieties, 463
*Rotylenchus brachyurus* pathogenicity on, 347
*Stemphylium* **floridanum** in, 11
wildfire resistant varieties, 463
toxin, mode of action, 659; **illuss. 600-663**
Tomato anthracnose, foliage, inoculum source, 338; **illuss. 340, 342-344**
bacterial canker, anatomy of infected plants, 267; **illuss. 268-270**
*Cattleya* Fusarium wilt in, 599; **illus. 601**
collar rot, air temperature effect, 168; **illuss. 169, 171**
crown gall, antibiotics, inhibition, 618; **illuss. 621, 623, 624**
damping-off control, 347
early blight, anthracnose, 340
fruit tumor, etiology, control, 132; **illus. 133**
Fusarium wilt, enzyme role, 381
nutrient sprays, 443
pectic enzymes role in, 79; **illus. 82**; 524, **illus. 525**
resistance, 303
gray leaf spot, air temperature effect, 168; **illuss. 169, 171**
histology, 654; **illuss. 655-657**
late blight, breeding for resistance, 186
leaf spot, *Stemphylium* **floridanum** sp. nov., 11, **illuss. 12, 13**
nailhead spot, air temperature effect, 168; **illuss. 169, 171**
*Phytophthora infestans* races, potato, relationship, 473
resistance to races, breeding, 464
*Pseudomonas solanacearum*, pathogenicity of isolates, 348
*Pyrenochaeta terrestris* in, 350
ring spot virus biophysical characteristics, electrophoresis, electron micrographs, 334; **illuss. 335, 336**
soybean bud-blight incitant, 500; **illus. 501**
spotted-wilt virus, techniques, review, 208; **illus. 214**
TMV on seed, 636
tobacco anthracnose in, 465
TOOLE, E. RICHARD, 177
*Torulopsis*, fungus culture, 466
Toxin production, *Helminthosporium victoriae*, 453; **illuss. 456, 457**
TRESHOW, MICHAEL, 132
*N*-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, see Fungicides, captan
*Trichoderma*, fungus culture, 466
*Trichodorus* sp., in cotton, 349
$\alpha\alpha$-Trithiobis(*N*-dimethylthioformamide), see Fungicides, Crag 5400
TRUE, R. P., 466
Tucker, Clarence Mitchell, biographical sketch, 351, **illus.**
Turf brown patch control, 186
nematode control, 350
snow mold control, 59; **illus. 61**
Turnip yellow mosaic virus, electron microscopy, **illuss. 189, 192, 194**
*Tylenchorhynchus* spp., see under Nematodes
*Typhula itoana* in turf, control, 59; **illus. 61**
Tyrothricin, *Agrobacterium tumefaciens* and gall tissue, 618

Ultraviolet, filipin assay, **illus. 560**
radiation, *Stemphylium solani* sporulation, 141; **illuss. 142, 143**
*Uncinula necator* control, 695

*Uromyces phaseoli*, chemicals in infected leaves, 43, **illus. 45, 46**
*Ustilago kolleri*, haplo-lethal deficiency, 521, **illus.**
*maydis*, quantitative cyclone spore collector method, 240
*nuda*, longevity in barley seed, 637
physiologic races, 73
*U. tritici*, spore germination study, 585; **illuss. 586-588**
*tritici*, wheat inheritance of resistance, 8
wheat varieties reaction, 513; **illus. 514**
*zeae*, sexual compatibility studies, 370; **illus. 372**

*Vaccinium* spp., wild, blueberry stunt virus sources, 465
VALLEGA, JOSÉ, 242
*Valsa kunzei*, conifer canker, relation to, 686; **illus. 688**
var. **kunzei** var. nov., 689
var. **piceae** var. nov., 689
Vancide, see under Fungicides
VAN DER PLANK, J. E., (222)
Vapam, see Fungicides, Stauffer N-869
Variegation, strawberry, 464
VARNEY, E. H., (465)
VAUGHAN, EDWARD K., 56
V-C 1-13, see under Nematocides
VENKATARAM, C. S., 240
*Venturia inaequalis*, resistance to fungicides, tests, 467; **illus. 469**
VERGARA, CLAUDIO, (695)
*Verticillium albo-atrum*, avocado susceptibility, 635
*albo-atrum*, fungicide tests, 398; **illus. 401**
light, response to, 465
longevity, 180
rose rootstock infections, 695
soil fumigation, 696
organisms, antagonistic, 696
strawberry resistance, 387; **illus. 388**
cotton seedlings, control, 350
sp. antibiotic tests in Elm, 185
soil-water and sapwood-water content related to, in maple, 184
toxins produced by, 183
Vinca, blueberry stunt virus, 465
Viomycin, soil, production and role, 407
Viruses, general, assay techniques, review, 196
chemical assay, 196
colorimetry, 196
dry-weight determination, 196
electron microscopy, 187; **illuss. 189, 190, 192, 194, 196**
generation time, 696
insect-transmitted, techniques, review, 208
vector inoculation, 196
labile, purification techniques, 208
mechanical inoculation, 196
*Prunus* spp., anthocyanosis and carbohydrate content of leaves, 184
purification, 196
serology, 196
spectrophotometry, 196
stream double refraction, dilution end-point, 196
Viruses, almond bud failure in peach, 323; **illuss. 325, 326**
apple green mottle, 186
mosaic in peach, 292; **illus. 293**
barley yellow dwarf, Holland, 695
bean mosaic, a gladiolus—similar to, 696
southern mosaic, noformicin effect, 281
seed maturation effect, 17
thiouracil effect, 281
blackberry mosaic complex, 272; **illus. 273**
blueberry stunt, wild sources, 465
carnation mosaic, 464
ringspot, 464
Cattleya leaf necrosis, Cymbidium mosaic, 327; **illuss. 328, 329**
celery-yellows, leafhopper transmission to aster, 185
Viruses—*continued*
cherry buckskin, leafhopper vectors, 694
"freckle fruit," 696
necrotic ring spot, *Prunus tomentosa* index plant, 320; **illuss. 321-323**
chrysanthemum aspermy, 2; **illus. 5**
flower distortion, 2; **illus. 5**
mosaic, 2; **illus. 5**
citrus tristeza, yellows and pitting complex, 222
cucumber mosaic, cowpea resistance, 563
mosaic, resistant varieties, 451
southern, insect transmission, 217; **illus. 218**
curly top, water pimpernel reaction and recovery, 531; **illuss. 532, 535**
Cymbidium mosaic hosts, electron microscopy, 327; **illuss. 328, 329, 331**
fig mosaic mite transmission, 52, **illus. 53, 54**
a gladiolus—similar to bean mosaic, 696
lettuce mosaic, aphid transmission, 357; **illus. 368**
seed transmission, 63; **illuss. 65, 67**
oats yellow dwarf, Holland, 695
pea streak, aphid, 665, **illus.**
electron microscopy, **illus. 189**
peach apple mosaic in, 292; **illus. 293**
"mule ear" from almond bud failure, 323; **illuss. 325, 326**
rosette, antibiotics, chemotherapy, 348
*Prunus tomentosa* index plant, 320; **illuss. 321-323**
western X, yellow leaf roll, interference, 694
yellow bud mosaic, soil, retention by, 696
yellow leaf roll, celery host, 694
leafhopper transmission, celery, 694
western X interference, 694
potato leafroll, reaction of starch from cells, 185
S, latent virus related to, 693
X immunity, 445; **illuss. 447-449**
immunity, clonal lines, 313; **illuss. 316, 318**
latent virus related to S, 693
nutrition, *Nicotiana* spp., 567; **illus. 570**
temperature effect in *Nicotiana* spp., 493; **illuss. 494-496**
Y, varieties reaction to, 148
yellow-dwarf, techniques, review, 208; **illus. 214**
prune dwarf, *Prunus tomentosa* index plant, 320; **illuss. 321-323**
squash mosaic, 3 components in, 145
electron microscopy, **illus. 192**
sedimentation patterns, **illus. 146**
stone fruit ring spot, cherry, 565
PLMV, purification, 574; **illus. 575**
strawberry free and non-free plants, 183
vein banding, 307; **illus. 309**
sugar beet yellows, mechanical transmission, 233; **illus. 234**
sugar cane mosaic, electron microscopy, 694
sweet potato internal cork, elimination, 185
tobacco mosaic, assay techniques, 196
mosaic, calcium deficiency effect, 288
chemicals in leaves, 43; **illus. 45**
Cymbidium mosaic, compared in *Datura stramonium*; **illus. 331**
early time course of, 695
electron microscopy, **illuss. 189, 192, 194**; 603; **illuss. 604-606**
*Nicotiana glutinosa*, 695
noformicin effect, 281
organic acids effect on, 291
proteins, 696
resistant varieties, 463
strains, 696
temperature of infection, effect, 595
thiouracil effect, 281
tomato seed, 636
ringspot, assay techniques, 196

Viruses, tobacco ringspot—*continued*
composition of leaves, 506
nutrition, cucumber, 567; **illus. 570**
soybean bud-blight incitant, 500; **illus. 501**
tomato ring spot, biophysical characteristics, electrophoresis, electron micrographs, 334; **illuss. 335, 336**
spotted-wilt, techniques, review, 208; **illus. 214**
turnip yellow mosaic, electron microscopy, **illuss. 189, 192, 194**
wheat mosaic, electron microscopy, 348
a winter rye for identifying, 637
*Aceria tulipae*, spread, control, 116; **illuss. 117-120**
wound-tumor, techniques, review, 208; **illus. 214**
yellow-net, Solanaceous hosts, 579, **illus.**
Vitamins, see under Nutrition and Nutrition of microorganisms
Volcani, Zafrira, (613)

Waggoner, Paul E., 79
Wagnon, H. Keith, (323), 696
Walker, J. C., (145), (303), (381), (443), 451, (524), (563)
Wallace, J. M., (52)
Walnut branch-wilt, sunburn effect, control, 607; **illuss. 608, 609, 611**
Water pimpernel curly top virus, reaction and recovery, 531; **illuss. 532, 535**
Waterman, Alma M., 686
Watermelon Fusarium wilt, 435; **illus. 436**
Weather, hot dry, associated with potato internal brown spot, 184
Webb, R. E., 579, 635
Weber, George F., (11)
Weeks, T. E., (574)
Weihing, John L., (595)
Weiss, Freeman A., 466
Wheat bunt, antagonism of root rotting organisms, 288
bunt, chlamydospore formation, **illus. 432**
inoculation methods, 480
resistance, environmental factors, 428; **illuss. 430, 432, 433**
calcium sulfamate effect, 503
loose smut, inheritance of resistance, 8
varieties reaction, resistance, 513; **illus. 514**
mosaic virus, electron microscopy, 348
a winter rye for identifying, 637
*Puccinia graminis* var. *tritici*, heterocaryosis basis for variation, 639; **illuss. 641, 643**
root-rotting organisms, antagonism to bunt, 288
rust races in South America, 242; **illuss. 243, 244**
seedlings, Tilletia infection, **illus. 430**
snow mold control, 696
Wheat—*continued*
stem rust control, 503
streak mosaic, *Aceria tulipae*, spread, control, 116, **illuss. 117-120**
Wheeler, H. E., (453)
Whitehead, Marvin D., 350, (413)
Whitfield, George B ,(559)
Wilcoxson, Roy D., (639)
Wildman, S. G., (695)
Wilhelm, Stephen, 180, (272), 387, (635), (695), 696
Williams, A. S., 290, (348)
Williams, H. E., 696
Wilson, E. E., 323
Wilson, J. B., 473
Wilson, W. F., (348)
Wolf, E. A., (291)
Wolf, Frederick A., 506
Wolf, Frederick T., 350, (506)

*Xanthomonas campestris*, cabbage inheritance of resistance, 35; **illus. 36**
*campestris* in cabbage varieties, 55
medium for, 461
*geranii X. pelargonii*, relationship, 613; **illus. 616**
*malvacearum*, bacteriophage attacking, 49; **illus. 50**
*pelargonii X. gerani*, relationship, 613; **illus. 616**
*phaseoli*, resistance to, testing method, 519; **illus. 520**
*pruni*, bacteriophage production, 580
time of infection related to spring canker, 285
spp., *Bacterium herbicola*, antagonism, 466
strains, bacteriophages specificity, 596; **illus. 597**
*vesticatoria* in pepper, control, 183
Xanthone, *Fusarium oxysporum* var. *nicotianae*, production, by, 350
*Xiphinema* spp., see under Nematodes
X-ray, oats stem rust, resistance, mutation, 490

Yanagihara, I., (238)
Yarwood, C. E., 43, 696
Yeast, sugar cane rot, antibiosis, 37, **illus. 38**
Young, D. W., 350

Zaumeyer, W. J., 466
Zentmeyer, George A., 109, 398, 635
Zerlate, see Fungicides, Ziram
Zinc dimethyldithiocarbamate, see Fungicides, ziram
ethelenebis [dithiocarbamate], see Fungicides, zineb
oxide, antifermentative for culture of *Panagrellus redivivus*, 184
Zineb, see under Fungicides
Ziram, see under Fungicides
Zscheile, F. P., (428), 480
*Zygosaccharomyces prioranus*, filipin, 559

# What Plant Food Means To The Grower

Plant food, properly used, is one of the cheapest aids to success a grower can employ. It will save him labor by growing his crops on fewer acres; increase yields by thickening and strengthening the stand of crops and making them more disease-resistant; improve the quality to obtain top prices; and prevent loss of his soil assets by preventing erosion and maintaining fertility. In order to produce as efficiently as possible, the grower is turning more and more to the agricultural scientists for the results of research and practical information on the use of plant food.

## American Potash Institute, Inc.

**1102 Sixteenth St., N. W.** **Washington 6, D. C.**

*Member Companies: American Potash & Chemical Corporation • Duval Sulphur & Potash Company • Potash Company of America • Southwest Potash Corporation • United States Potash Company*

# TRIARCH PRODUCTS

## FINE MICROSCOPE SLIDES FOR DISCRIMINATING BIOLOGISTS

As we prepare and sell only microscope slides, we are able to give you better quality, better service and better prices than you can get elsewhere.

**Remember, TRIARCH means ACCURACY, DEPENDABILITY, SERVICE**

***Write for our illustrated catalog No. 10-A***

**GEO. H. CONANT,** **RIPON, WISCONSIN**

# BIOLOGICAL ABSTRACTS

***Covers the World's Biological Literature***

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all the biological literature from some 2,000 journals, *Biological Abstracts* also is published in five low-priced sectional editions to meet the needs of individual biologists. Section D (Plant Sciences) affords a very complete coverage of Phytopathology and related fields—list price, $21.00 (less 20% discount to non-profit, educational institutions, and 50% to individuals). Foreign, 50 cents postage additional. Write for full information and a sample copy.

**BIOLOGICAL ABSTRACTS**
**University of Pennsylvania**
**Philadelphia 4, Pa.**



# THE NEW CLOTH-BOUND PHYTOPATHOLOGICAL CLASSIC

No. 9. TARGIONI TOZZETTI. True nature, causes, and sad effects of the rust, the bunt, the smut, and other maladies of wheat and oats in the field. 1767. Translated from the Italian by L. R. Tehon. Price $2.00.

Price of Classics Nos. 3 to 9 inclusive, $7.50.

See PHYTOPATHOLOGY VOL. 45, NO. 3, 1955 for complete listing.

*Please remit with order.*

**Published by the**

**AMERICAN PHYTOPATHOLOGICAL SOCIETY**
L. CARL KNORR, *Editor*

Address orders to

C. W. Boothroyd, Department of Plant Pathology

Cornell University, Ithaca, New York

The Flue-cured grower who takes the proper precautionary steps to control nematodes can produce vigorous, healthy plants of high quality.

TOBACCO growers in the Flue-cured region have good reason to dread infestations of their crop by nematodes. These tiny, worm-like creatures live in the soil, attacking and feeding upon tobacco roots. They interfere with root development and also prevent roots from functioning normally. Since roots serve as life lines to the tobacco, damage by nematodes means a stunting and weakening of the whole plant. Once a field becomes infested with nematodes, there is no practical method of eliminating them completely. However, there are effective control measures, such as the practice of crop rotation, and in some cases, soil fumigation. Certain cropping sequences that have proved helpful in reducing nematode populations have been worked out by Federal and State Agricultural Experiment Stations.

# Reduce *Nematode Population* with Rotation Practices

per crop rotations *reduce the root-knot natode population* in the soil. This fall is not too late to start a suitable rotation, the growers who put in fall crops that fit a nematode-control program have taken first step toward better crops in the fu-. Crops that will help control nematodes crops that will make the problem worse listed elsewhere on this page. Further in-mation on rotations for nematode control appear in this series next summer.

hree distinct kinds of nematodes are now wn to cause serious root damage to to-co: (1) root-knot, (2) meadow or root n (brown root rot) and (3) stunt. Al-gh these nematodes differ in their feed-habits, the above-ground symptoms they duce are similar. Affected plants are ted, appear to be starved, and wilt on hot . The leaves fire around the edges or in and ripen prematurely.

nderground, the symptoms produced by hree kinds of nematodes are different. -knot nematodes enter the young roots e they become embedded in the soft tis-They remain in one place and suck the ices. The affected roots become swollen, acing the typical knots which later de-Meadow nematodes enter the roots and through the soft tissues feeding upon plant cell contents. These tissues are producing the small, brown lesions arly known as brown root rot. The stunt tode does not enter the root, but at-s itself to the surface of the root, pierces d sucks out cell juices. This results in root development.

nether the trouble is caused by root-knot, low, or stunt nematodes, the result is to the Flue-cured grower. Roots are le to obtain the necessary amounts of and nutrients from the soil, and the ed plants often die before harvest. The ged condition of the roots also increases ptibility to other disease organisms.

ce varieties with adequate resistance ematodes are not yet available, the r should safeguard his crop by preven-ractices.

## ROTATIONS FOR ROOT-KNOT

Rotation is helpful in controlling root-knot nematodes only if the crops rotated with tobacco are resistant to the disease. Harvested peanuts, corn, weeds, small grains, cotton, and crotalaria have been used successfully in many parts of the Flue-cured region on light, sandy soils. Crotalaria, however, should never be used immediately preceding the tobacco crop. Certain rotations tested by Agricultural Experiment Stations have resulted in reduced root-knot damage; four-year rotation of cotton, corn, peanuts, and tobacco; three-year rotation of peanuts, oats-weeds, and tobacco; three-year rotation of corn, oats-weeds, and tobacco; three-year rotation of cotton, peanuts, and tobacco; and two-year rotation of small grains-weeds and tobacco. In the shorter rotations, it is advisable to change the alternate crops frequently rather than repeat the same sequence over and over again.

Some crops, because of their susceptibility to nematodes, should never be used in tobacco rotations. These include cowpeas, peppers, Ladino clover, Irish potatoes, Austrian winter peas, tomatoes, lespedeza, and soybeans.

Although rotations used to control root knot are not always effective against meadow and stunt nematodes, they still offer considerable protection. Proper rotations not only help prevent disease, but build up the fertility and tilth of the soil for all crops.

## OTHER CONTROL METHODS

Soon after harvest, the tobacco stalks should be cut and the roots turned up. Expose as much root surface to the sun as possible with a turning plow, and then drag over them a few days later with a section harrow or weeder. This practice prevents the nematode eggs from over-wintering in the soil. After exposed roots have completely dried, the field may be prepared for a small grain cover crop.

Other precautions and preventive practices can be used to help prevent build-up of nematodes. These are primarily intended to keep the organism from spreading, and include sterilization of plant beds, the transplanting of healthy plants only, and cultivation by the ridge method to keep surface water under control.

Recommended soil fumigants are effective in reducing nematode populations quickly, and can be used to give practical control in badly infested soils. Fumigation should take place only when the soil's moisture content is moderate—neither very wet nor very dry. If the soil is right, the grower may fumigate any time up to two weeks before transplanting.

Work is under way in an effort to develop Flue-cured varieties which will have resistance to nematodes, but it has been difficult to combine high resistance with good quality and satisfactory yield.

For more information on nematode diseases, get in touch with your County Agent, other Extension Workers, or Vocational Agriculture Teachers.

These two plants were set the same day. The startling difference in size shows the power of nematodes to sap tobacco's growth. The plant at the left is healthy, but the plant at the right was attacked by nematodes.

*The American Tobacco Company*

Copyright 1955

-cured advertisement appearing in farm publications. It is representative of the series started in the Burley area and later extended to the Flue-cured area.

# tolerances established by F.D.A. for Stauffer CAPTAN residues!

A tolerance of 20 parts per million has been established by the F. D. A. for residues of CAPTAN (N-trichloromethyl mercapto-4-cyclohexene-1,2-dicarboximide) in or on:

| | | | |
|---|---|---|---|
| apples | grapes | peaches | quinces |
| apricots | lemons | pears | summer squash |
| cantaloups | limes | peppers | strawberries |
| cherries | mangoes | pineapple | tangerines |
| cucumbers | nectarines | plums | tomatoes |
| eggplant | oranges | pumpkin | watermelon |
| grapefruit | | | winter squash |

Under normal conditions:

APPLES & PEACHES
may be sprayed with 2 lb. Stauffer Captan 50-W per 100 gallons up to harvesting without exceeding residue tolerance.

CHERRIES, GRAPES
and other small fruit may require a minimum of five days between spraying and picking.

Recent expansion of Stauffer's production facilities insures an adequate supply of Captan 50-W for all agricultural applications through the 1956 growing season.

Detailed information on CAPTAN and other STAUFFER products is available, and samples are offered for experimental work. In the East, write to: Technical Director, Agricultural Chemical Sales, Stauffer Chemical Company, Chauncey, N. Y. In the West, address inquiries to: Director of Agricultural Research, Stauffer Chemical Company, Mountain View, California.

Stauffer CHEMICALS SINCE 1885

## STAUFFER CHEMICAL COMPANY

380 Madison Avenue, New York 17, N. Y.

*"STAUFFER—Since 1885, a dependable name!"*